MAGNESIUM SALTS VOL II #62
COPIES OF ARTICLES CITED

18E

Magnesium Salts-Volume 2 #62 9/21/73

VOLUME 2

GRAS MONOGRAPH SERIES

MAGNESIUM SALTS

(COPIES OF ARTICLES CITED IN MONOGRAPH SUMMARY)

prepared for

THE FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION
AND WELFARE

SEPTEMBER 21, 1973

prepared by

Tracor Jitco, Inc.

Arch Derm & Syp 41: 707-710 (1940)

FIXED ERUPTION FROM MAGNESIUM HYDROXIDE

POLYSENSITIVITY

E. WILLIAM ABRAMOWITZ, M.D.
NEW YORK
AND
JOSEPH J. RUSSO, M.D.
ALBANY, N. Y.

The erythematous pigmentary fixed eruption first reported by Louis Brocq [1] in 1894 was definitely shown to be due to antipyrine. The lesions flared up each time the drug was taken. Since then this type of eruption and others of the urticarial, erythematous and eczematous group, recurring in situ, have been reported following the use of acetophenetidin (phenacetin), acriflavine hydrochloride (trypaflavine), aminopyrine and its compounds, antimony and potassium tartrate, the arsenicals (acetylarsan, arsphenamines, mapharsen and tryparsamide), barbiturates, bismuth salts, cinchophen (atophan), iodides, ipecac, emetine, ipomea (scammony), isacen (diacetyldihydroxyphenylisatin), mercury, phenolphthalein, quinine and the salicylates (acetylsalicylic acid and sodium salicylate). Other agents, such as vaccines, liquors, psychic stress, physical exertion, menstruation and autotoxic substances, have also been implicated.[2] Urbach [3] reported a fixed eruption from the eating of lentil soup, and Cooke [4] observed one which followed the eating of tomatoes. Bowen [5] described a similar eruption from a dental powder containing indian, or karaya, gum, while Loveman and Simon [6] showed sulfanilamide to be responsible for eruptions also.

Chargin [7] noted polysensitivity in several persons who presented fixed eruptions due to arsphenamine. The lesions flared up after the

1. Brocq, L.: Eruption érythémato-pigmentée fixe due à l'antipyrine, Ann. de dermat. et syph. 5:308, 1894.
2. Abramowitz, E. W., and Noun, M. H.: Fixed Drug Eruptions, Arch. Dermat. & Syph. 35:875 (May) 1937.
3. Urbach, E.: Ueber fixe nutritiv-allergische Exantheme, Klin. Wchnschr. 15:1208 (Aug. 22) 1936.
4. Cooke, R. A.: Personal communication to the authors.
5. Bowen, R.: Karaya Gum as a Cause of Urticaria, Arch. Dermat. & Syph. 39:506 (March) 1939.
6. Loveman, A. B., and Simon, F. A.: Fixed Eruption and Stomatitis Due to Sulfanilamide, Arch. Dermat. & Syph. 40:29 (July) 1939.
7. Chargin, L.: Fixed Eruption Due to Arsphenamine, Arch. Dermat. & Syph. 37:144 (Jan.) 1938; Fixed Eruption in a Patient Sensitive to Arsphenamine and Phenolphthalein in Different Areas, ibid. 38:474 (Sept.) 1938.

001

administration of antipyrine, various arsenicals, a bismuth preparation or phenolphthalein, and in 1 case new fixed lesions appeared.

In view of these reports, it appears that the cause of a fixed eruption is not limited to a few organic compounds but that any drug may provoke such an eruption. Agents even of a drugless nature may produce such cutaneous manifestations. The explanation that a benzene ring is responsible for a fixed eruption appears inadequate, for bismuth hydroxide and mild mercurous chloride, both inorganic compounds, are known to cause similar lesions.[2]

The following report of a fixed eruption from magnesium hydroxide is interesting, as it shows that first one organic drug and then another and finally a simple inorganic compound caused the same type of eruption in the same patient.

REPORT OF A CASE

Mrs. M. P., a private patient aged 32, was American born, white and the mother of two healthy children. There was no history of allergy. She had always been well except for occasional constipation, for which she obtained relief by taking an emulsion of liquid petrolatum containing agar-agar and phenolphthalein. She never observed any ill effects from its use but discontinued it the latter part of 1938.

The eruption first appeared in 1936, after ingestion of a single dose of phenobarbital, which apparently had been taken for the first time. The cutaneous lesions appeared on the extensor surface of the middle finger and the hypothenar area of the right hand and the little finger of the left hand. They were described as red spots of varying size that left a slight pigmentation. No recurrence developed until 1937, when a rash reappeared in the same areas after the use of the same drug.

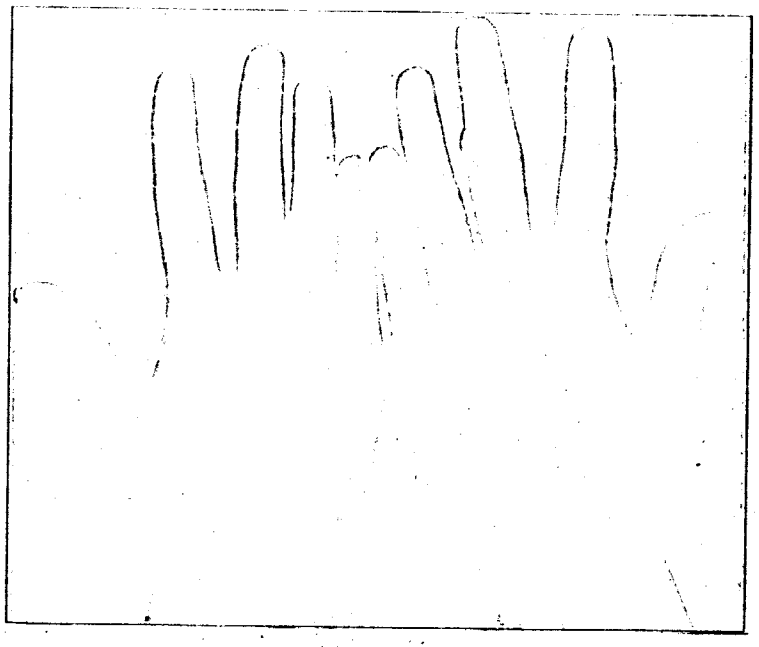
A flare-up of the eruption in the previously involved areas appeared on Feb. 12, 1938, after the patient had taken three daily doses of a confectionary tablet containing phenolphthalein. On February 26, when the eruption had subsided, the identical eruption reappeared eight hours after the taking of 1 grain (0.06 Gm.) of phenobarbital. No further recurrence developed until December. She then began to use a well known brand of magnesium hydroxide in tablet form, taking 1 tablet daily for seven days. The eruption appeared after the last dose and was similar to the previous outbreaks. The same result occurred later when one of us (J. J. R.) asked the patient to take the tablets of magnesium hydroxide again and also when tablets of another brand of the drug were used.

The patient then avoided all medication until July 22, 1939, when she was directed to take 2 tablets of magnesium hydroxide each day. The recurrence developed about six hours after the last dose on the third day. A similar outbreak developed later, after magnesia magma U. S. P. (suspension of magnesium hydroxide in water) was ingested.

In order to test her sensitivity to other drugs she was given 5 grains (0.32 Gm.) of acetylsalicylic acid. One week later she received daily doses of 5 grains (0.32 Gm.) of antipyrine, but no reaction developed after ingestion of either drug. During the investigation attention was given to the elimination of anything that might possibly contain phenolphthalein or any other drug. All mouth washes, dentifrices, colored candy, cakes and ice cream were strictly avoided. The makers

of the tablets of magnesium hydroxide, on request, stated that the composition of these tablets was magnesium hydroxide, starch, sugar and oil of peppermint. As the suspension of magnesium hydroxide (magnesia magma) contained none of the ingredients in the tablets except the drug itself, no further testing was done.

The eruption has always been the same, consisting, after the first outbreak of the recurrence in situ, of a circumscribed purplish erythema about the size of a silver dollar located on the hypothenar eminence of the right hand, with a smaller but similarly colored patch on the extensor surface of the middle finger of the right hand and the little finger of the left hand. The eruption is accompanied by itching and usually clears up in two or three weeks, leaving a faint pigmentation. With the taking of the tablets of magnesium hydroxide beginning



Three days after the outbreak of a fixed bullous eruption on the middle finger of the right hand and erythema on the right hypothenar eminence which followed the ingestion of tablets of magnesium hydroxide.

on July 22, the patch on the right palm appeared more hemorrhagic, and on the middle finger of the right hand appeared a large bulla. The patient's hands were photographed on July 27, 1939, three days after the outbreak. Consent for a biopsy could not be obtained.

The patient was certain that the eruption never appeared except when she took one dose of phenobarbital, three doses of phenolphthalein or several doses of magnesium hydroxide.

Recurrence in previously affected areas, even though varying from an erythema to a bullous lesion, is characteristic of the so-called "fixed" eruption. The presence of polysensitivity is indicated by the flare-up after ingestion of phenobarbital, phenolphthalein or magnesium hydroxide.

SUMMARY AND CONCLUSIONS

A case is reported in which a fixed eruption followed the ingestion of tablets of magnesium hydroxide, an inorganic substance. The lesions first appeared after the ingestion of phenobarbital, later after that of phenolphthalein and finally after the taking of magnesium hydroxide.

Apparently almost any agent, chemical or otherwise, simple or complex, inorganic or organic, may cause this peculiar manifestation in the skin.

J. Appl. Physiol. 15: 503-507 (1960)

Magnesium metabolism in human beings: studies with Mg^{28} [1]

JERRY K. AIKAWA,[2] GERALD S. GORDON[3] AND ELOISE L. RHOADES (With the Technical Assistance of Dale R. Harms and Jacqueline Z. Reardon)
Department of Medicine, University of Colorado School of Medicine, Denver, Colorado

AIKAWA, JERRY K., GERALD S. GORDON AND ELOISE L. RHOADES. *Magnesium metabolism in human beings: studies with Mg^{28}.* J. Appl. Physiol. 15(3): 503-507. 1960.—Mg^{28} was used to explore the kinetics of magnesium distribution in 9 normal individuals and in 16 patients with various diseases. When Mg^{28} was given in 12-30 mEq of stable magnesium intravenously, plasma disappearance was rapid within the first several hours. In normal subjects a mean of 19.8% of the injected radioactivity was accounted for in the urine within 24 hours. Fecal excretion was negligible, although equilibration of Mg^{28} in bile occurred within 18 hours. The specific activities of plasma and urine stabilized by the 18th hour, and showed only a gradual decrease thereafter. Exchangeable magnesium contents in normal subjects ranged between 2.6 and 5.3 mEq/kg of body weight—less than 16% of the estimated total body content of magnesium. Mg^{28} exchanged very slowly with the stable ion in bone, muscle and erythrocytes. The results in patients with diabetes mellitus and hepatic diseases showed no striking differences from those obtained in normal subjects.

MAGNESIUM, an intracellular cation present in quantities second only to potassium, is of considerable importance in living organisms (1). It is known to activate many enzymes (2), to play an essential role in neuromuscular function and to be an important constituent of bone. Until recent years, studies on magnesium metabolism have been hampered by technologic problems: chemical determinations of magnesium in biologic material were difficult, and no suitable isotope was available for dynamic studies. Mg^{28}, a radioactive isotope discovered in 1953 by Sheline and Johnson (3), has recently become available in quantities and with a specific activity high enough for observations to be made in human subjects without the danger of chemical toxicity.

Received for publication October 5, 1959.
[1] This work was supported in part by Contract AT(11-1)-282 between the University of Colorado and the U.S. Atomic Energy Commission and in part by a grant-in-aid from the American Heart Association and the Colorado Heart Association.
[2] Established Investigator of the American Heart Association.
[3] Public Health Service Research Fellow.

In the present study Mg^{28} (supplied by Brookhaven National Laboratory on allocation from U.S. Atomic Energy Commission) was used to explore the kinetics of magnesium distribution in normal subjects and in patients with various diseases. The results of similar studies in rabbits, and of a study on the gastrointestinal absorption of Mg^{28} in man and in rabbits have been reported previously (4-6).

MATERIALS AND METHODS

A total of 27 studies were performed on 25 hospitalized subjects (17 males and 8 females) between the ages of 12 and 76 years (table 1). No attempt was made to control the patient's caloric or fluid intake during the period of observations; none, however, received any medication containing magnesium.

Radiomagnesium (Mg^{28}) was prepared in a manner described previously (6) and administered intravenously in 250-350 ml of 5% dextrose in water. The average amount of carrier magnesium administered was 13.5 mEq together with about 10^7 c.p.m. of radioactivity.

Urine samples and samples of blood from the brachial vein were collected at intervals over the next 24 hours. In a few instances, blood samples were collected for as long as 37 hours and urine samples for 67 hours. All samples were collected in chemically clean glassware, and the blood samples were drawn into heparinized syringes.

In addition to the routine sampling performed on all patients, the following additional specimens were obtained in selected cases: *a*) blood samples taken simultaneously from the brachial artery and vein during and after infusion in three patients; *b*) samples of liver, muscle, fat, skin, appendix, bone or bile from four patients; and *c*) complete 72-hour stool collection in one patient. In three patients, surface monitoring was performed with a directional scintillation counter attached to a recording counting rate meter.

Serum and urine magnesium determinations were performed by a modification (7) of the molybdivanadate method for phosphate (8).

Samples of serum, urine and tissue were assayed for

TABLE 1. *Summary of Results of Mg^{28} Studies in Human Subjects*

Case No.	Age	Sex	Wt.	Dose of Mg	Clinical Diagnosis	Infus. Time	Serum Mg Concen. B.L.[a]	Max.[b]	Min.[c]	Renal Excretion Of Mg^{28} 0–8′	0–24′	Of Mg^{24} 0–8′	0–24′	Exchangeable Mg[d] Plasma	Urine	Mean
			kg	*mEq*		*min.*	*mEq/l.*							*mEq*		*mEq/kg*
1	17	F	49.5	14.0	Normal	6	1.68	4.11	1.29	24.3	26.7	9.5	13.5	125	135	2.8
2	18	M	100.7	30.0	Normal	83	1.51	2.27	1.34	18.9	20.6	18.1	22.4	270	309	2.6
3	49	M	65.2	97.0	Normal	75	1.32	5.49	1.91	42.4	45.0[f]	70.4	79.0[f]		186	2.8
				16.0		60	1.91	2.61	1.21	26.2	30.7	20.6	31.6			
4	21	M	71.8	13.0	Normal	60	1.56	2.08	1.36	18.7	20.2	5.3	6.0	397	370	5.3
5	54	F	52.3	14.5	Normal except for cholecystitis	51	1.61	2.35	1.53	19.5	20.8	6.0	10.6	146	135	2.7
6	47	M	67.5	20.0	Normal	19	1.64	3.15	1.54	16.6	19.6	1.9		229	259	3.6
7	36	M	56.6	12.0	Normal	16	2.10	2.70	1.90	16.2	25.3	15.6	36.0	222	262	4.3
8	52	M	69.8	12.0	Normal	13	1.70	2.50	1.80	9.7	11.9	6.1	10.5	255	249	3.6
9	36	M	63.2	12.0	Normal	17	1.60	2.50	1.60	17.7	20.7	17.7	24.4	181	217	3.1
10	72	M	74.1	18.7	Diabetes mellitus	23		2.22	1.55	8.9	14.2	1.1	8.2	194	177	2.5
11	22	M	45.5	10.0	Diabetes mellitus	16	1.27	1.78	1.29	11.6	13.6	8.4	12.4	268	241	5.6
			48.6	8.0		18		1.50	1.50	12.0	17.4	16.3	23.7	254	247	5.2
12	50	F	56.4	10.0	Diabetes mellitus	5	1.52	1.99	1.45	19.2	23.4	9.5	18.0	222	198	3.7
13	12	M	33.6	7.5	Diabetes mellitus	10	1.41		1.39	19.9	22.4	19.9	22.4	157	161	4.7
14	76	M	53.2	14.0	Ca pancreas, biliary obst.	60	2.56	2.64	1.75	7.0	12.5	4.1	10.4	156	147	3.8
15	52	F	53.6	20.0	Cirrhosis	20	1.13	2.80	1.51	43.7	55.4	7.3	14.9	27	39	0.1
16	21	M	72.7	13.0	Hepatitis	20		2.31	1.64	22.6	26.2	22.1	32.0	234	239	3.3
17	62	F	66.8	10.0	Hepatitis	8	1.53	1.93	1.37	9.4	12.6	6.2	10.3	93	105	1.5
18	43	F	67.4	18.7	Hepatitis	19	1.27	2.01	1.32	11.3	12.6	8.7	17.7	209	208	3.1
19	46	F	60.0	10.0	Cushing's	7	1.90	2.67	1.90	14.6	17.8	10.9	14.8	181	176	3.0
20	16	M	61.4	8.0	Leukemia	18	1.92	2.24	1.83	9.6	12.2	7.0	27.5	320	342	5.4
21	50	M	113.2	12.0	Pickwickian	25	1.41	2.10	1.58	9.5	13.5	8.2	15.5	337	314	2.9
22	23	F	54.1	14.0	Epilepsy	5	1.10	3.18	0.72	24.3	30.0	14.8	25.5	146	175	3.6
23	15	M	46.8	13.7	Hodgkin's	50	1.07	1.74	0.68	20.8	23.9	12.4	18.8	168	194	3.9
24	17	M	57.3	13.7	Renal tuberculosis	34	1.30	1.96	1.09	20.5	26.1	12.8	21.4	250	227	4.2
25	40	M	89.5	28.0	Hypertensive cardiovasc. dis.	42	1.25	2.25	1.38	9.4	11.7	15.6	25.3	422	448	4.9

[a] Value before infusion of Mg^{28}. [b] Maximum plasma Mg concentrations were always observed at the end of an infusion of Mg^{28}. [c] Minimum values observed within 34–37 hours after completion of infusion of Mg^{28}. [d] Expressed as % of Mg^{28} administered intravenously. [e] Values calculated from specific activities of plasma and urine obtained 24 hours after completion of Mg^{28} infusion. [f] Cumulative values from 0 to 14 hours.

gamma ray activity with a Nuclear model DS-3 well scintillation counter connected to a Tracerlab Superscaler. A total of at least 10,000 counts were made on each sample. All determinations were corrected for physical decay.

For calculation of specific activity: specific activity of urine, serum, bile and tissue specimens $= \frac{\text{c.p.m./ml}}{\text{mEq/l.}}$

$$\text{Relative specific activity} = \frac{\text{specific activity of tissue}}{\text{specific activity of serum}}$$

The exchangeable magnesium content was determined by use of the usual formula employed for calculation of exchangeable electrolyte content (6).

RESULTS

Studies in Normal Subjects

Ten studies were performed in nine normal subjects. From 12 to 30 mEq of tagged magnesium were given intravenously over a period of 6–90 minutes. Rapid infusion produced a sensation of burning along the course of the brachial vein up to the shoulder. One patient (*case 22*) experienced a sensation of generalized heat when a concentrated solution of magnesium was administered very rapidly; the serum magnesium concentration at this time was 3.18 mEq/l. One subject (*case 3*) was initially given, over a period of 75 minutes, 97 mEq of magnesium in 1000 ml of a 3% solution of dextrose in water. Toward the end of the infusion, coincident with the insertion of an arterial needle, extreme drowsiness, tachycardia and hypotension developed. The serum magnesium level at the time of this reaction was 5.5 mEq/l.

The mean serum magnesium concentration prior to the administration of magnesium was 1.64 mEq/l (table 1). At the end of the magnesium infusions, the serum concentrations ranged between 2.3 and 5.5 mEq/l. In seven of nine instances, subsequent values for serum magnesium fell below the base-line values, usually between 17 and 35 hours after completion of the injection.

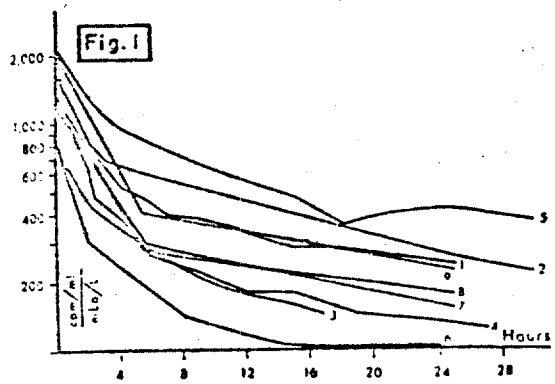


FIG. 1. Specific activities of plasma specimens in normal subjects given Mg^{28} intravenously.

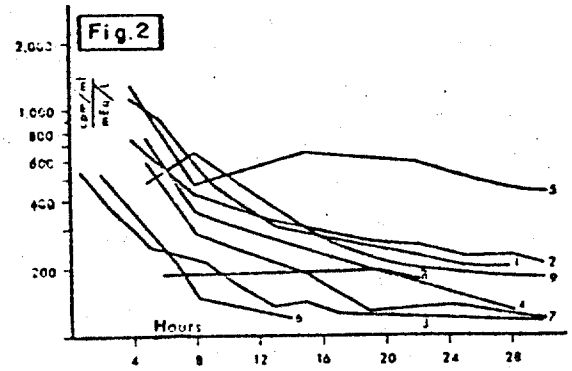


FIG. 2. Specific activities of urine specimens in normal subjects given Mg^{28} intravenously.

one subject (*case 4*), however, showed the lowest value at 90 minutes.

Plasma disappearance of Mg^{28}. The plasma disappearance curves of the nine normal subjects were similar in appearance, showing a rapid initial decrease in concentration for the first 6–8 hours, followed by a slower decline which extended through at least 30 hours. In *case 3* an infusion of 97 mEq of magnesium was given 14 hours before the second infusion of 16 mEq; the second clearance curve was similar to those of the other subjects not given a prior infusion.

volume of fluid available for dilution of the in- Mg^{28} was calculated from the plasma concentrations of radioactivity. Between 1.25 and 4.75 hours (mean = 3.5 hours) after completion of the intravenous infusions, the radiomagnesium space exceeded the calculated volume of total body water.

Renal excretion. When 12–30 mEq of magnesium were given, 9.7%–26.2% (mean = 19.8%) of the infused radioactivity was recovered in the urine within 8 hours, and 11.9%–30.7% (mean = 21.8%) was recovered within 24 hours. In the subject who received 97 mEq of magnesium (*case 3*), 42.4% was recovered within 8 hours.

In the 24-hour period after administration of the radioactive material, renal excretion of stable magnesium (Mg^{24}) approached or exceeded the amount of magnesium given intravenously. The lowest excretion value (6 mEq) was found in *case 4*, the subject with the highest value of exchangeable magnesium content (5.3 mEq/kg). The subject given 97 mEq of magnesium (*case 3*) excreted 45.0% of the injected radioactivity and 79.4 mEq of stable magnesium within 14 hours.

Specific activity. The results of serial determinations of the specific activity of plasma and urine samples in normal subjects are shown graphically in figures 1 and 2. The curves are in general similar to the configurations obtained for the plasma disappearance of Mg^{28}. Specific activities fluctuated less in plasma than in urine. The specific activities decreased gradually in both urine and ma after 18 hours.

xchangeable magnesium content. Values calculated from ne urine obtained at 24 hours agreed rather closely with those calculated from plasma; the range in the normal subjects was 2.6–5.3 mEq/kg of body weight, with a mean of 3.4 mEq/kg.

Other studies in normal subjects. In three patients (*cases 2, 3* and *4*) serial blood specimens were obtained simultaneously from the brachial artery and vein during the infusion and for 2.5 hours after completion of the infusion of Mg^{28}. No significant differences were noted in the concentrations of radioactivity from the two sources.

Fecal excretion. In *case 4*, 0.62% of the radioactivity administered intravenously was recovered in the stool within 24 hours; an additional 1.17% was recovered in the next 48 hours.

External survey. In two subjects serial external surveys of radioactivity over the entire body were carried out. In one subject (*case 6*) the maximal distribution of radioactivity at the end of infusion was over the right upper quadrant of the abdomen. In the other subject so studied (*case 4*) the highest counting rates at the end of the infusion were found anteriorly over the liver and the kidneys. Three hours later, the highest distributions were at the femoral triangular areas; at 9 hours, the radioactivity was the same over the epigastrium, the umbilicus, and the suprapubic and femoral triangular areas. At 24 hours, highest counting rates were detected over the epigastrium and umbilical areas, while radioactivity over the femoral triangles had decreased. Radioactivity over the long bones and skull was negligible.

Tissue biopsy studies. An elective cholecystectomy and an incidental appendectomy were performed on one subject (*case 5*) 9 hours after the infusion of Mg^{28}. Specimens of liver, periappendiceal fat and appendix were obtained at operation, and serial plasma and bile specimens were collected postoperatively. The specific activities of serum and bile are given in table 2. The relative specific activity in bile reached unity at 18 hours. At 9 hours the concentration of Mg^{28} in the serum was 1032 c.p.m/ml; in the liver, 8453 c.p.m/gm (wet weight) of tissue; in the appendix, 4209 c.p.m/gm; in fat, 608 c.p.m/gm.

Studies in Patients With Diseases

Diabetes mellitus (cases 10–13). Five studies were performed in four patients with diabetes mellitus. One pa-

TABLE 2. *Specific Activities of Plasma and Bile in Patient Given 14.5 mEq Mg Intravenously*

Time After Injection of Mg^{28}, hr.	Specific Activity		Relative Specific Activity Bile/Serum
	Plasma	Bile	
9	670	154	0.23
12	557	522	0.94
15	475	434	0.91
18	356	384	1.08
21	395	462	1.17
26.5	399	330	0.83
30	369	434	1.18

tient (*case 11*), during acidosis and coma, had a low serum magnesium value of 1.27 mEq/l. The plasma clearance curves observed in this subject during acidosis and coma and a week later were similar.

The mean values for cumulative renal excretion of radioactivity at 8 hours (14.2 %) and 24 hours (18.2 %) were slightly lower than the respective mean values observed in normal subjects. In four of five instances the urinary excretion of stable magnesium during the first 24 hours exceeded the amount of Mg^{28} given intravenously.

The lowest value for exchangeable magnesium content in this group (2.5 mEq/kg) was found in the subject (*case 10*) who showed the flattest Mg^{28} plasma disappearance curve and the most rapid attainment of stable specific activities in urine and plasma. The highest value (5.6 mEq/kg) was in the diabetic patient studied during acidosis and coma (*case 11*).

One subject (*case 10*) had gangrene of the right leg. Amputation was performed below the knee 10 hours after an intravenous infusion of Mg^{28}. Samples of the fibula, tibia, tibial bone marrow, skin, muscle and subcutaneous connective tissue were assayed for radioactivity content and magnesium content. Comparison of the specific activities of the tissues showed that at 10 hours the injected Mg^{28} had not reached equilibrium with the magnesium in any of the tissues studied. The fibula and tibia showed the lowest relative specific activities, with values of 0.007 and 0.003, respectively.

Hepatic diseases (*cases 14–18*). Five patients with hepatic diseases were studied. Initial serum magnesium values were very low in two patients (*cases 15* and *18*).

Mg^{28} plasma clearance curves and urine and serum specific activities were no different from those observed in normal subjects. In two patients (*cases 14* and *17*) urinary excretion of radioactivity was low during the first 8 hours, but within the normal range at 24 hours. The urinary excretion of stable magnesium during this period was within the range found in normal subjects. One patient (*case 15*) had a very high 24-hour urinary excretion of Mg^{28} (55 %). This same patient, who died 6 weeks later in hepatic coma, showed the lowest exchangeable magnesium content found in this study (0.1 mEq/kg), as well as a low initial plasma magnesium concentration. Another patient (*case 17*) had an exchangeable magnesium value of 1.5 mEq/kg.

External survey in *case 16* at 7, 12 and 16 hours after infusion of Mg^{28} showed the greatest distribution of radioactivity over the liver and the next highest counting rate over the kidneys. Radioactivity over the bones was extremely low.

Other disease states (*case 19–25*). One patient with idiopathic epilepsy (*case 22*), one with renal tuberculosis (*case 24*) and one with hypertensive cardiovascular disease who was being actively treated (*case 25*) had serum magnesium levels below 1.30 mEq/l. The plasma clearance curves for Mg^{28}, specific activities of urine and plasma, and renal excretion of Mg^{28} and stable magnesium were all within the ranges found in normal subjects. Exchangeable magnesium values ranged between 2.9 and 5.4 mEq/kg; the highest value was found in a patient with acute lymphatic leukemia (*case 20*).

Serial specimens of washed erythrocytes were assayed for radioactivity content in four subjects (*cases 20, 23–25*). No radioactivity was detected in blood samples obtained up to 24 hours after the administration of Mg^{28}.

COMMENT

The low specific activity of the Mg^{28} currently available makes impossible true tracer studies of magnesium metabolism. Because of the low concentration, we were unable to follow plasma and urinary radioactivity with confidence beyond 36 hours. The doses of stable magnesium (12–30 mEq) usually administered intravenously, however, were considerably smaller than the loading dose of 97 mEq which produced a systemic reaction. For this reason, we feel that the data obtained in the present study may reflect the metabolism by the usual mechanisms of an extra load of magnesium rather than the chemical toxicity of this cation.

The results in normal subjects show that the stable magnesium infused with Mg^{28} was very rapidly cleared from the extracellular fluid. The intravenous administration of as much as 30 mEq of magnesium did not cause systemic reactions as long as the rate of infusion was slow enough so that the plasma concentration of magnesium did not exceed 3.0 mEq/l. The volume of fluid available for the dilution of this ion, as calculated from the plasma concentrations of Mg^{28}, exceeded the volume of total body water within a few hours. Since the amount of Mg^{28} excreted in the urine during this period was a small fraction of the injected dose, renal excretion alone could not account for this rapid clearance of Mg^{28} from the blood. The data obtained from tissue biopsies show concentrations of Mg^{28} in liver, appendix, fat, skin and subcutaneous connective tissue which cannot be attributed solely to the relative extracellular composition of these tissues. All of these observations suggest that Mg^{28} rapidly entered cells.

From previous studies (9, 10), in which large doses of magnesium were administered parenterally and deductions made from changes in the concentrations of stable magnesium in the blood or from the urinary excretion of this ion, it was concluded that magnesium was initially confined to the extracellular fluid and that excretion of the parenterally administered material was for the most part complete in 24 hours. A fraction of the

administered magnesium was thought to be bound to tissues. Our results suggest that, when 12–30 mEq of magnesium are infused slowly into normal subjects, 70% or more is retained in the body for at least 24 hours. The fact that the urinary excretion of stable magnesium during this time approximates the amount infused probably led previous investigators to the erroneous interpretation that most of the infused magnesium was rapidly excreted by the kidneys. Our data show that the infusion of a fairly large amount of magnesium results in a compensatory renal excretion of the body store of magnesium, and that the material excreted is not necessarily the ions which were administered.

After about 18 hours the specific activities in plasma and urine showed only a slight gradual decrease. These results suggest that the infused material had equilibrated in a rather labile body pool of magnesium, and that further exchange was occurring very slowly in a less labile pool. The size of the labile pool in normal subjects ranged between 135 and 397 mEq (2.6–5.3 mEq/kg of body weight). Previous analyses of entire adult human carcasses (11) have revealed the total body content of magnesium to be of the order of 1200–2400 mEq (27.2–35.1 mEq/kg of body weight). Thus, it appears that less than 16% of the total body content of magnesium is being measured by the present Mg^{28} exchange technique. These results contrast with those obtained in rabbits (6), in which species the values for exchangeable magnesium closely approximated the total carcass content of magnesium.

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 fact that the Mg^{28} concentration was the same in samples of arterial and venous blood taken from the forearm is additional evidence for the slow exchange of magnesium in bone and muscle.

It is known that magnesium metabolism is related to that of phosphorus and glucose (12). Because the liver is so important in carbohydrate metabolism, and diabetes is so intimately related to carbohydrate metabolism, the results of the present study with Mg^{28} in patients with hepatic disease or diabetes mellitus are of particular interest.

A low serum magnesium concentration was found in the patient with the severest case of diabetes mellitus, who was acidotic at the time of the initial study. Mg^{28} was removed from plasma more rapidly than in any other subject studied and the cumulative renal excretion of Mg^{28} was rather low, although still within the normal range. The value for exchangeable magnesium in this patient (5.6 mEq/kg of body weight) was the highest found in this study. Similar results were obtained a week later, when the diabetes was under good control, except that plasma clearance was slower and the urinary excretion of stable magnesium was increased. The significance of these changes is not entirely clear. Studies in three other patients with diabetes gave results which did not differ from those obtained in normal subjects.

Mg^{28} studies in five patients with liver disease suggest that clinical evidence of magnesium deficiency does not occur until the liver has been severely damaged. The patient (*case 15*) who had the lowest initial serum concentration, together with a low value for exchangeable magnesium and high urinary excretion of Mg^{28}, died soon afterward in hepatic coma. It would seem that only a minimal amount of functional hepatic tissue is necessary to retain magnesium in the body. Another patient (*case 17*) had an exchangeable magnesium content of only 1.5 mEq/kg, but showed no clinical evidence of this abnormality.

Results obtained in patients with a variety of other diseases gave no definite clues concerning the factors regulating the metabolism of magnesium.

Our current knowledge concerning magnesium metabolism in human beings may be briefly summarized as follows: when magnesium is introduced into the body by the gastrointestinal route, the fraction absorbed is considerably smaller than had been suggested previously (1). The factors regulating absorption across the gastrointestinal mucosa are not well understood, nor do we know how magnesium is transported from the portal system to the extracellular environment and eventually to the individual cells. It is not clear whether magnesium metabolism is dependent on a specific hormonal regulation. It appears to be related to the metabolism of phosphorus and glucose, and the liver seems to play a role. The labile pool of magnesium in the body, which is but a small fraction of the total body content of magnesium, is confined primarily to the soft tissues of the body. Magnesium exchange in bone, muscle and erythrocytes is extremely slow. Magnesium administered parenterally is excreted by the kidneys very slowly, but the over-all balance is maintained by a compensatory rapid excretion of endogenous magnesium.

REFERENCES

1. Duncan, G. G. *Diseases of Metabolism* (4th ed.). Philadelphia: Saunders, 1959, p. 245.
2. Lehninger, A. L. *Physiol. Rev.* 30: 393, 1950.
3. Sheline, R. K. and N. R. Johnson. *Phys. Rev.* 89: 520, 1953.
4. Aikawa, J. K., E. L. Rhoades and G. S. Gordon. *Proc. Soc. Exper. Biol. & Med.* 98: 29, 1958.
5. Aikawa, J. K. *Proc. Soc. Exper. Biol. & Med.* 100: 239, 1959.
6. Aikawa, J. K., E. L. Rhoades, D. Harms and J. Z. Reardon. *Am. J. Physiol.* 197: 99, 1959.
7. Aikawa, J. K. and E. L. Rhoades. *Am. J. Clin. Path.* 31: 314, 1959.
8. Simonsen, D. G., L. M. Westover and M. Wertman. *J. Biol. Chem.* 169: 39, 1947.
9. Smith, P., A. Winkler and B. Schwartz. *J. Biol. Chem.* 129: 51, 1939.
10. Wallace, G. and B. Brodie. *J. Pharmacol. & Exper. Therap.* 72: 44, 1941.
11. Widdowson, E. M., R. A. McCance and C. M. Spray. *Clin. Sc.* 10: 113, 1951.
12. Stutzman, F. L. *Studies in Magnesium Metabolism.* Ann Arbor, Mich.: Univ. Microfilms, 1952.

Clin. Sci., 1962, 22, 185–193

INTER-RELATION OF CALCIUM AND MAGNESIUM ABSORPTION

By N. ALCOCK* and I. MacINTYRE

(*From the Department of Chemical Pathology, Postgraduate Medical School, Ducane Road, London, W.*12)

Submitted May, 1961

A RELATIONSHIP between calcium and magnesium metabolism has been evident since 1909 when Mendel and Benedict showed in animals that an infusion of calcium produced an increase in the urinary excretion of magnesium and *vice versa*. In experimental magnesium deficiency (Tufts and Greenberg, 1938; Watchorn and McCance, 1937) disturbances in calcium metabolism including the production of hypercalcæmia (MacIntyre and Davidsson, 1958) have been reported.

Specific factors such as protein intake (McCance, Widdowson and Lehmann, 1942), the administration of growth hormone (Hanna, Harrison, MacIntyre and Fraser, 1961), large doses of vitamin D (Hanna, 1961) and of antibiotics (Heggeness, 1959) have been shown to influence magnesium absorption from the gut; however, no single factor has been shown to play a role in the absorption of magnesium similar to that of vitamin D in calcium absorption (Nicolaysen, 1937). The site of absorption of magnesium in man (Graham, Cæsar and Burgen, 1960) and in animals (Aikawa, 1959) appears to be the proximal part of the small intestine where calcium is also absorbed most effectively (Lindquist, 1952).

These considerations suggested that an investigation of the inter-relation of calcium and magnesium absorption would be worth while. We chose to do this by studying calcium absorption in the gut of magnesium deficient rats; a similar study of the effect of calcium deficiency on magnesium absorption was made. The renal excretion of calcium and magnesium in each group was also investigated.

METHODS

Four experiments with rats of initial weight between 90 and 110 grams were carried out. In three of these—experiments 1, 3 and 4—female animals were used, while in experiment 2 males were used. All of the animals were fed diets which were the same except for the magnesium or calcium content. In experiments 1, 2 and 3 magnesium was omitted from the diet of the experimental group of animals, while in experiment 4 calcium was omitted. In all experiments a normal control group of animals received a diet with both calcium and magnesium present in normal amounts. The rats were tube fed and after varying periods of deficiency balance studies were made on animals from each group (Hanna and Alcock, 1961). The period of magnesium deficiency was twenty three days in experiment 1, eleven days in experiment 2 and seventeen days in experiment 3. Calcium deficiency was continued for eighteen days in experiment 4.

Diet

The normal control diet and the magnesium deficient diet were the same as those used by MacIntyre and Davidsson (1958). The calcium deficient diet was the same as the normal diet except that calcium was omitted from the salt mixture, 25 grams of which was added per kilogram of diet used; the calcium, magnesium, sodium, potassium and phosphorus content of the respective diets

* Present address, The Rockefeller Institute, New York 21, N.Y.

is shown in Table 1. Chromium sesquioxide (50 mg. per rat per day) which is unabsorbed by the gut (Schürch, Lloyd and Crampton, 1950) was added to the respective diets three days prior to the collection of fæcal specimens. This was continued throughout the experimental period. The diet was prepared by adding sufficient distilled water to the dry components to form a smooth paste. The cod liver oil and the arachis oil, together with the water soluble vitamins were then added. The volume was adjusted by adding distilled water so that one gram of diet was suspended in a total volume of one millilitre. Each rat was fed 8 grams of diet per day. The daily intake of calcium and magnesium is shown in Table 2.

TABLE 1

The electrolyte content of the diets

(Magnesium, calcium, potassium and sodium content is expressed in terms of m-equiv./kg. of diet; phosphate content is expressed in terms of m-moles/kg. of diet.)

Type of diet	Mg	Ca	K	Na	Phosphate
Normal	34	300	145	45	100
Mg deficient	0·5	300	145	45	100
Ca deficient	34	3·0	145	45	100

TABLE 2

The daily intake (μ-equiv./rat) of calcium and magnesium

Diet	Magnesium	Calcium
Normal Control	272	2400
Magnesium Deficient	4	2400
Calcium Deficient	272	2

Method of feeding

The animals were tube fed throughout the entire course of each experiment. A small metal gag placed in the mouth to depress the tongue facilitated feeding. An all glass graduated syringe with a piece of rubber catheter tubing attached was used for feeding; the hole in the tip of the syringe barrel was widened to a diameter of 3 mm. so that a diet of fairly thick consistency could be fed. Four ml. of diet were given at 9.00 a.m. and 4·0 ml. at 5·00 p.m. daily. Distilled water was available *ad libitum* for drinking.

Balance periods

At the beginning of each balance period the animals were placed in individual metabolic cages (Hanna and Alcock, 1961) which allowed the separate collection of fæcal and urine specimens. In each experiment specimens were collected during 24-hour periods for the measurement of fæcal and/or urinary excretion of calcium and magnesium.

Blood specimens

At the conclusion of the balance periods the animals were anæsthetized by an intraperitoneal injection of 6 mg. of sodium pentobarbitone ("Nembutal"; Abbott Laboratories Ltd., London) —except in experiment 2 where ether was used—and blood was taken from the aorta for analysis.

Analytical methods

Calcium and magnesium were estimated in plasma, urine and fæces flame spectrophotometrically (MacIntyre, 1957; Alcock, MacIntyre and Radde, 1961). Chromium sesquioxide concentration in the fæcal specimens was estimated by the method of Whitby and Lang (1960). Creatinine in plasma was estimated by the method described by Owen, Iggo, Scandrett and Stewart (1954) and urine creatinine as described by King and Wootton (1956).

Glomerular filtration rate

The method used for estimating glomerular filtration rate in experiment 2 was based on that of Corcoran (1952). The glomerular filtration rate was calculated by measuring creatinine clearance. In view of the low concentration of creatinine in the plasma of the rat, creatinine (50 mg.) was injected

intraperitoneally prior to determining the clearance. At the same time 8·0 ml. of water was given by stomach tube. After 50 minutes the rat was made to urinate. During the following 10 minutes the animal was placed in a metabolic cage and urine was collected. At the end of this period the bladder was emptied again and the urine specimen added to the 10 minute collection. The rat was then quickly anæsthetized with ether and a specimen of blood collected.

RESULTS

Magnesium deficiency

The classical signs of magnesium deficiency (Kruse, Orent and McCollum, 1932; Watchorn and McCance, 1937; Greenberg and Tufts, 1938) were evident in the magnesium deficient rats. The time of onset and the duration of hyperæmia and vasodilatation varied from one batch of animals to another; convulsive seizures were common and the more severe occurrences caused death. As the deficiency progressed diarrhœa was occasionally evident but was not severe.

Fæcal excretion

Magnesium

In experiments 2 and 3 the fæcal excretion of magnesium was measured at intervals from 9 to 17 days after the commencement of the deficiency. The mean daily excretion in the magnesium deficient animals was 31·6 and 23·0 μ-equivalents in experiments 2 and 3 respectively. In the normal control animals the mean daily excretion of magnesium was 127·0 μ-equivalents in male animals (experiment 2) and 139·0 μ-equivalents in female animals (experiment 3). A summary of the results is given in Table 3.

TABLE 3

**Mean daily fæcal excretion of calcium and magnesium (μ-equivalents)*

	Excretion			
	Magnesium		Calcium	
Experiment No.	Experimental Group	Normal Group	Experimental Group	Normal Group
1	—	—	1658 (12) ± 101·6	2094 (12) ± 54·8
2	31·6 (21) ± 8·1	127·0 (29) ± 10·2	1394 (21) ± 111·7	1574 (29) ± 102·2
3	23·0 (28) ± 2·3	139·0 (26) ± 7·6	1649 (28) ± 76·7	1740 (26) ± 68·3
4	84·9 (26) ± 6·4	154·0 (28) ± 6·3	6·2 (26) ±0·95	1684 (28) ± 57·4

*Results are expressed as mean (number of determinations) ± S.E.M.

Calcium

The mean daily fæcal calcium excretion in the two groups of animals is shown in Table 3. The fæcal excretion of calcium by rats receiving the magnesium deficient diet was less than that of the normal control group in each of the three experiments. On combining the results from all three experiments and applying an analysis of variance described by Snedecor (1934) the difference in fæcal calcium excretion was shown to be significant at the 1% level ($F^1_{122} = 8·4$). The individual results are shown in a scatter diagram in Figure 1.

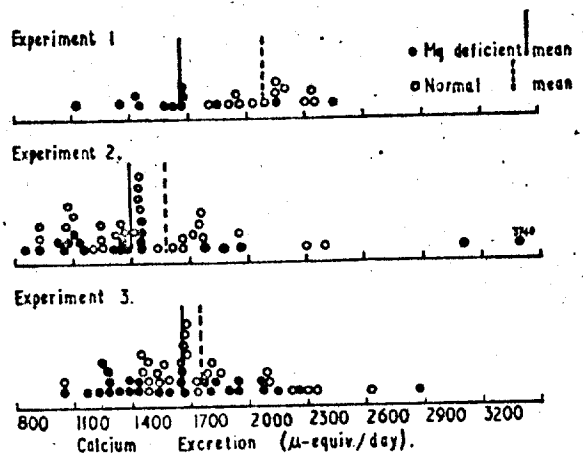


Fig. 1. Fæcal calcium excretion in magnesium deficient and normal control rats.

Urinary excretion

The urinary excretion of magnesium and calcium was measured from the first day of magnesium deficiency in experiment 2 (Table 4). In magnesium deficiency the urinary excretion of magnesium fell markedly during the first day and was negligible from the third day onwards as shown in Figure 2a. Calcium excretion also was diminished during the first 24 hours and was significantly less than in the normal group by the end of the third day ($p < 0 \cdot 025$). The results are shown in Figure 2b. Diminished calcium excretion continued throughout the period of magnesium deficiency despite the presence of hypercalcæmia.

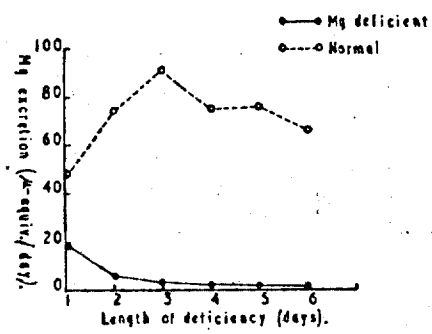


Fig. 2a.

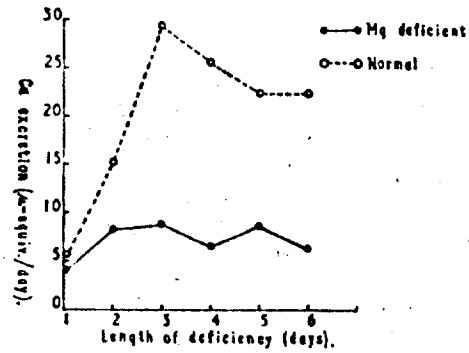


Fig. 2b.

Fig. 2a. Urinary magnesium excretion in magnesium deficient and normal control rats.
Fig. 2b. Urinary calcium excretion in magnesium deficient and normal control rats.

Plasma

The mean plasma calcium level estimated at the conclusion of the balance periods was significantly increased to 6·1 m-equiv./litre in the magnesium deficient animals ; the normal mean was 5·6 m-equiv./litre . The mean plasma magnesium concentration was 0·73 m-equiv./litre compared with 1·6 m-equiv./litre in the normal control group.

Glomerular filtration rate

The glomerular filtration rate calculated as the creatinine clearance on the 11th day of magnesium deficiency in experiment 2 was 1·24 ml./min. (S.E.M. 0·08) and that in the normal animals 1·49 ml./min. (S.E.M. 0·40); the difference was not significant.

Calcium deficiency

The animals on the calcium deficient diet showed no abnormal signs apart from the fact that the stools were soft and less bulky than normal. The fæcal dry weight in these animals was markedly diminished.

Fæcal excretion

The fæcal excretion of calcium by the calcium deficient animals was negligible during the period studied (16–18 days). The excretion of magnesium was markedly diminished. The mean daily excretion of magnesium and calcium together with that of the normal control animals is shown in Table 3; individual magnesium results are shown diagramatically in Figure 3.

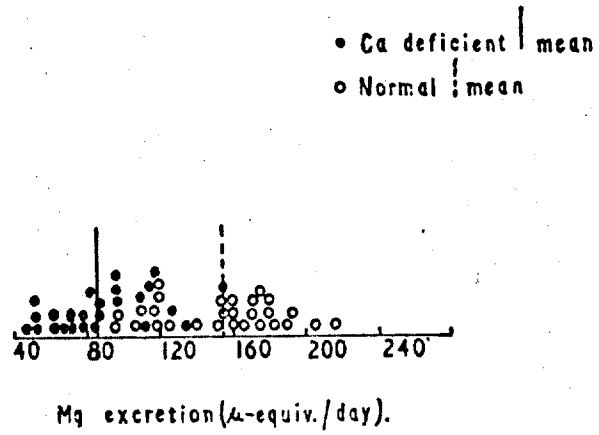


Fig. 3. Fæcal magnesium excretion in calcium deficient and normal control rats.

TABLE 4

**Mean daily urinary excretion (μ-equivalents) of magnesium and calcium.*

		Excretion					
		Magnesium			Calcium		
Experiment No.	Length of deficiency (days)	Experimental Group	Normal Group	p	Experimental Group	Normal Group	p
1	16–23	1·9 (22) ± 0·2	51·1 (23) ± 4·4	<0·001	12·6 (22) ± 1·8	32·7 (23) ± 4·1	<0·001
2	1–6	5·2 (30) ± 1·2	71·9 (30) ± 4·0	<0·001	7·1 (30) ± 0·5	20·0 (30) ± 3·2	<0·001
3	7–10	2·0 (46) ± 0·2	81·6 (48) ± 3·0	<0·001	6·7 (46) ± 0·3	19·3 (48) ± 1·9	<0·001
4	16–18	140·5 (36) + 3·5	94·3 (35) + 5·3	<0·001	6·1 (36) ± 0·4	32·4 (35) ± 3·8	<0·001

* Results are expressed as mean (number of determinations) ± S.E.M.

Urinary excretion

The urinary excretion of calcium during the period studied showed that in the absence of calcium from the diet, this ion is conserved by the kidney (Table 4). A marked increase in the urinary magnesium output resulted from the increased absorption of magnesium from the gut of the animals which were deprived of calcium.

Plasma

The plasma calcium concentration did not differ between the two groups ; the mean values were 5·7 m-equiv./litre in the normal group and 5·6 m-equiv./litre in the calcium deficient group. The plasma magnesium concentration in the experimental group was slightly higher (1·8 m-equiv./litre) than in the normal group (1·6 m-equiv./litre). The difference, however, was not statistically significant.

Discussion

A number of factors are known to affect the absorption of calcium from the gut. Its enhancement by vitamin D is well established (Nicolaysen, 1937 ; Albright and Reifenstein, 1948a ; Lindquist, 1952 ; Migicovsky, 1957). It has also been suggested that the parathyroid glands influence calcium absorption in the intestine (Robertson, 1942 ; Albright and Reifenstein, 1948b ; Lichtwitz and Parlier, 1959). Other specific factors known to increase calcium absorption are increased protein intake (McCance *et al*, 1942), L-lysine and lactose (Wasserman, Comar, Schooley and Lengemann, 1959), neomycin (Heggeness, 1959) and L-arginine (Wasserman, Comar and Nold, 1956). Increased protein intake and neomycin had the same effect on magnesium absorption as on calcium absorption.

The absorption of both calcium and magnesium will be impaired by substances which form insoluble compounds with the respective ions, e.g. phytic acid, fatty acids and excess phosphate. Thus the results obtained by O'Dell, Morris and Regan (1960) can be explained by the depression of magnesium absorption due to the presence of excess phosphate.

Tufts and Greenberg (1938) showed that increasing the calcium content of their diet accentuated magnesium deficiency in the rat. Similarly, further addition of calcium to the diet of milk-fed calves depressed the plasma magnesium level still more (Allcroft, personal communication). The intravenous injection of calcium has been shown to increase the urinary excretion of magnesium and *vice versa* (Mendel and Benedict, 1909 ; Chesley and Tepper, 1958 ; Womersley, 1956 ; Barker, Elkinton and Clark, 1959). An *in vitro* relationship between calcium and magnesium absorption was found by Schachter and Rosen (1959) who showed that calcium transport across the intestinal wall in rats, rabbits and guinea pigs was depressed by the presence of magnesium ions in the medium.

TABLE 5

Daily absorption (μ-equivalents) of calcium and magnesium from the gut in magnesium deficiency

Experiment		Absorption		
		Calcium	Magnesium	Total
2	Experimental group	1006	—	1006
	Normal group	826	145	971
3	Experimental group	752	—	752
	Normal group	660	133	793

In the present work, an increased plasma calcium level in magnesium deficiency in the rat has been shown to be associated with an increase in the absorption of calcium from the gut. The total calcium absorbed by the animals which were deprived of magnesium was found to be almost identical with the net calcium plus magnesium absorbed by the normal control animals (Table 5).

Our results strongly suggest the existence of a common transport mechanism for the transfer of the divalent cations, calcium and magnesium, across the intestinal wall. This would also explain the findings of Tufts and Greenberg (1938), Allcroft (personal communication) and Schachter and Rosen (1959). Our finding that the absence of calcium from the diet increased magnesium absorption from the gut is further evidence in support of the existence of a common transport mechanism. A similar mechanism may exist in the kidney ; the decrease in urinary calcium excretion by magnesium deficient rats, despite hypercalcæmia, could be explained by an increase in calcium absorption, due to a decreased magnesium load, by a tubular absorptive pathway common to calcium and magnesium. Although no detectable change in the glomerular filtration rate was demonstrated, the technique used was too crude to exclude altered glomerular filtration rate as an explanation of our results ; a small change in the filtration rate could account for the differences in excretion found.

It is possible that a common absorptive mechanism for calcium and magnesium may exist in many different species. Thus, MacIntyre, Hanna, Booth and Read (1961) record a case of steatorrhœa in whom a negative magnesium balance was enhanced by increasing the dietary calcium. Berglund and Forster (1958) suggested the existence of a transport system common to divalent cations to explain their findings in the aglomerular kidney of the marine teleost, Lophius americanus, where the divalent cation with the higher excretion rate depressed competitively transfer of the other ion. Our results also provide an explanation of hypomagnesæmia in the milk-fed calf as a conditional magnesium deficiency due to abnormally high calcium intake. This view is suggested by the work of Allcroft who found that the addition of calcium lactate to the milk hastened the appearance and increased the severity of hypomagnesæmia.

Summary

The effects of dietary magnesium and calcium deficiency on the absorption of calcium and magnesium, respectively, have been studied by balance techniques in rats of initial weight 90–110 grams.

In the gastro-intestinal tract, calcium absorption was increased in the absence of magnesium while, in the absence of calcium, magnesium absorption was increased.

In magnesium deficiency, urinary excretion of calcium was decreased. The decrease could be explained by increased tubular reabsorption of calcium.

The hypothesis is advanced that magnesium and calcium are absorbed by a common transport mechanism in both the intestine and the renal tubule.

We should like to thank the Medical Research Council for financial support. The work described in this paper is included in a Ph.D. thesis (University of London) by N.A.

REFERENCES

Aikawa, J. K. (1959). Gastrointestinal absorption of ^{28}magnesium in rabbits. Proc. Soc. exp. Biol. & Med., 100, 293.

Albright, F., and Reifenstein, E. C. (1948a). The parathyroid glands and metabolic bone disease. The Williams and Wilkins Co., Baltimore, p. 125.

ALBRIGHT, F., and REIFENSTEIN, E. C. (1948b). The parathyroid glands and metabolic bone disease. The Williams and Wilkins Co., Baltimore, p. 134.

ALCOCK, N., MACINTYRE, I., and RADDE, I. (1960). The determination of magnesium in bioligical fluids and tissues by flame spectrophotometry. J. clin. Path., 13, 506.

BARKER, E. S., ELKINGTON, J. R., and CLARK, J. K. (1959). Studies of the renal excretion of magnesium in man. J. clin. Invest., 38, 1733.

BERGLUND, F., and FORSTER, R. P. (1958). Renal tubular transport of inorganic divalent ions by the aglomerular marine teleost, Lophius americanus. J. gen. Physiol., 41, 429.

CHESLEY, L. C., and TEPPER, I. (1958). Some effects of magnesium loading upon renal excretion of magnesium and certain other electrolytes. J. clin. Invest., 37, 1362.

CORCORAN, A. C. (1952). Determination of inulin clearance. Methods in medical research. 5, 246. The Year Book Publishers, Inc. Chicago.

GREENBERG, D. M., and TUFTS, E. V. (1938). Nature of magnesium tetany. Am. J. Physiol., 121, 416.

GRAHAM, L. A., CAESAR, J. J., and BURGEN, A. S. V. (1960). Gastrointestinal absorption and excretion of ^{28}magnesium in man. Metabolism, 9, 646.

HANNA, S. (1961). The influence of vitamin D on magnesium metabolism. Metabolism, in the press.

HANNA, S., and ALCOCK, N. (1961). A technique for balance studies in the rat. Clin. Sci., 21, 285.

HANNA, S., HARRISON, M., MACINTYRE, I., and FRASER, R. (1961). Effects of growth hormone on calcium and magnesium metabolism. British med. J., in the press.

HEGGENESS, F. W. (1959). Effect of antibiotics on the gastrointestinal absorption of calcium and magnesium in the rat. J. Nutrition, 68, 573.

KING, E. J., and WOOTTON, I. D. P. (1956). Micro-analysis in medical Biochemistry (third edition). J. and A. Churchill Ltd. (London).

KRUSE, H. D., ORENT, E. R., and MCCOLLUM, E. V. (1932). Studies on magnesium deficiency in animals; 1. Symptomatology resulting from magnesium deprivation. J. biol. Chem., 96, 519.

LICHTWITZ, A., and PARLIER, R. (1959). Les parathyroides et le métabolisme phosphocalcique. La Presse Médicale, 67, 134.

LINDQUIST, B. (1952). Effect of vitamin D on metabolism of radio-calcium in rachitic rats. Acta pædiatrica, Supplement 86.

MCCANCE, R. A., WIDDOWSON, E. M., and LEHMANN, H. (1942). Effect of protein intake on absorption of calcium and magnesium. Biochem. J., 36, 686.

MACINTYRE, I. (1957). The flame spectrophotometric determination of calcium in biological fluids and an isotopic analysis of the errors in the Kramer-Tisdall procedure. Biochem. J., 67, 164.

MACINTYRE, I., and DAVIDSSON, D. (1958). The production of secondary potassium depletion, sodium retention, nephrocalcinosis and hypercalcæmia by magnesium deficiency. Biochem. J., 70, 456.

MACINTYRE, I., HANNA, S., BOOTH, C. C., and READ, A. E. (1961). Intracellular magnesium deficiency in man. Clin. Sci., 20, 297.

MENDEL, L. B., and BENEDICT, S. R. (1909). The paths of excretion for inorganic compounds: IV. The excretion of magnesium; V. the excretion of calcium. Amer. J. Physiol., 25, 1, 28.

MIGICOVSKY, B. B. (1957). Influence of vitamin D on calcium resorption and accretion. Canad. J. Biochem. and Physiol., 35, 1267.

NICOLAYSEN, R. (1937). Studies upon the mode of action of vitamin D; III. Influence of vitamin D on absorption of calcium and phosphorus in the rat. Biochem. J., 31, 122.

O'DELL, B. L., MORRIS, E. R., and REGAN, W. O. (1960). Magnesium requirement of guinea pigs and rats. Effect of calcium and phosphorus and symptoms of magnesium deficiency. J. Nutrition 70, 103.

OWEN, J. A., IGGO, B., SCANDRETT, F. J., and STEWART, C. P. (1954). The determination of creatinine in plasma or serum and in urine; a critical examination. Biochem. J., 58, 426.

ROBERTSON, J. D. (1942). Calcium and phosphorus excretion in thyrotoxicosis and myxœdema. Lancet, 1, 672.

SCHACHTER, D., and ROSEN, S. M. (1959). Active transport of Ca^{45} by the small intestine and its dependence on Vitamin D. Am. J. Physiol., 196, 357.

SCHÜRCH, A. F., LLOYD, L. E., and CRAMPTON, E. W. (1950). The use of chromic oxide as an index for determining the digestibility of a diet. J. Nutrition 41, 629.

SNEDECOR, G. W. (1934). Analysis of Variance, P. 52. Collegiate Press Inc., Ames, Iowa.

TUFTS, E. V., and GREENBERG, D. M. (1938). Biochemistry of magnesium deficiency; chemical changes resulting from magnesium deprivation. J. biol. Chem., 122, 693.

WASSERMAN, R. H., COMAR, C. L., and NOLD, M. M. (1956). The influence of amino acids and other organic compounds on the gastrointestinal absorption of calcium[45] and strontium[89] in the rat. J. Nutrition, 59, 371.

WASSERMAN, R. H., COMAR, C. L., SCHOOLEY, J. C., and LENGEMANN, F. W. (1957). Interrelated effects of L-lysine and other dietary factors on the gastro-intestinal absorption of calcium[45] in the rat and chick. J. Nutrition, 62, 367.

WATCHORN, E., and MCCANCE, R. A. (1937). Subacute magnesium deficiency in rats. Biochem. J., 31, 1379.

WHITBY, L. G., and LANG, D. (1960). Experience with the chromic oxide method of fæcal marking metabolic balance investigations on humans. J. clin. Invest., 39, 854.

WOMERSLEY, R. A. (1956). Studies on the renal excretion of magnesium and other electrolytes. Clin. Sci., 15, 465.

012

March 30, 1930

559

THE PROBLEM OF SALINE DRINKING WATERS

In the February 21, 1930, issue of Science, V. G. Heller and C. H. Larwood reported experiments on the deleterious effects of certain saline drinking waters. In the course of a ground-water survey of northwestern Minnesota a few years ago, the writer had opportunity to observe in the field similar effects from waters of moderate concentrations, mostly lower than those reported by Heller and Larwood. The worst waters appeared to be those rich in sulphates. The waters are commonly (though often incorrectly) referred to as "alkali" waters. The sulphates in some waters are accompanied by true alkali, *i.e.*, soda or potash, but lime and magnesia generally are more abundant. Some are associated with high chlorides and others are not.

These high sulphate waters in Minnesota are common in the till-plain just east of the glacial Lake Agassiz basin and in the lake-bed itself (now the Red River Valley). Thus nine representative samples taken from various depths and localities in Stevens County range in salinity from 664 to 2,800 p.p.m., and the average of the nine is 1,575 p.p.m. anhydrous salts. The minimum sulphate is 29 per cent., the maximum 58 per cent. and the average 49 per cent., or approximately 770 p.p.m. The averages of the other main constituents are: Ca 14 per cent., Mg 5 per cent., Na 11 per cent., K 2 per cent., CO_3 (including HCO_3, recalculated) 16 per cent., Cl 1 per cent. In four of the nine samples, Na is more abundant than Ca. The low amount of chloride is noteworthy.

In the Cretaceous waters which are tapped by drilling in the Red River Valley farther west, sulphates and chlorides (apparently connate) are both abundant. Thus water from a city well at Wheaton, Minnesota, shows Ca and Mg 1 per cent., Na 33 per cent., K 4 per cent., CO_3 4 per cent., SO_4 28 per cent. and Cl 30 per cent.; total salinity, 2,794 p.p.m. This is a typical soft salty water of the region. Similar waters occur in the basal sand of the Cretaceous rock system northward to the Canadian boundary, but with a salinity as high as 10,000 p.p.m. or more. Toward the east these waters are diluted and become sodium or calcium bicarbonate waters. The waters from the glacial drift above the Cretaceous beds are bicarbonate solutions of Ca or Na, but many show high sulphates also. Waters from wells ending in the clays of glacial Lake Agassiz are notoriously high in sulphates, so that they have a bitter taste and drastic purgative effect on the drinker. In one case a 12-inch bored well 45 feet deep furnishes water containing 2,104 p.p.m. SO_4 in a total salinity of 3,600 p.p.m. As an extreme, another 80-foot well furnishes water with 3,590 p.p.m. SO_4 in a total salinity of 5,756 p.p.m.

The drastic cathartic action and the weakening effect of these natural solutions of Epsom and Glaubers salts on man and on live stock are well known in the region. Some persons believe that disorders of kidneys and bladder also result, but that effect is not established. It seems true though that no matter how well they are fed, cattle and hogs can not be fattened for market while they drink such water. Indeed, the case is even worse. The cattle develop a run-down, ragged appearance and many eventually weaken and die prematurely. The principal difficulty in such cases seems to be that a degeneration of the bones sets in, so that most of the lime is abstracted from them. These bones are reduced to gristle that can be tied in knots and easily punctured with a knife. Calves are stunted in growth and many never mature at all. The cows develop strange appetites for bones, leather, wood, etc. The condition is alleviated but perhaps not cured by feeding bone-meal or ground limestone.

Altogether about one fourth of the State of Minnesota is affected in some degree by this problem. Furthermore, the observation of cattle from Dakota and Montana, where similar waters are known, suggests that the difficulty is really wide-spread but has not been properly diagnosed. With the present trend toward "diversified farming" involving cattle-raising and dairying, a considerable territory faces a critical problem. It seems probable that people using such waters are affected in the same way as cattle, but perhaps in different degree, so that questions of human physiology and diet also are involved.

It is to be hoped that the experimental work of Heller and Larwood will be continued, and that the effect on the equilibrium of body calcium may be studied in particular, because the problem has a large economic bearing.

Ira S. Allison

Oregon State College

[4] For example, C. N. Moore, *Bull. Amer. Math. Soc.*, 34: 414, 1928.

Proc. Soc. Exp Biol Med 25: 1928

ceeds the intake, and there is a shift of the major excretion from the stool to the urine.

3964

Antipyretic Action of Magnesium Chlorid Alone and Combined With Amidopyrin.

H. G. BARBOUR AND J. E. WINTER.

From the Department of Physiology and Pharmacology, School of Medicine, University of Louisville, Kentucky.

The temperature reducing properties of magnesium salts in doses below the threshold for the well known Meltzer narcosis, have been described by J. Schütz,[1] for normal rabbits. Rabbits artificially fevered with *β-tetra-hydro-naphthylamin* also exhibited antipyresis with magnesium. The latter, however, has apparently never been investigated hitherto with regard to the reduction of fever of bacterial origin. In animals with hay-infusion fever, it is not difficult to demonstrate a marked antipyretic action with doses of magnesium salts far below the toxic range. This can be especially well shown with magnesium chlorid in rabbits previously injected with hay-infusion incubated 48 hours, at 37° C.

Healthy adult rabbits after fasting over night were injected subcutaneously with 1 cc. (per kilo) hay-infusion. A rise in temperature amounting to at least 1.5° F. was found within about 2 hours, in approximately two-thirds of the injected rabbits. The antipyretic agent was injected subcutaneously, usually 2 to 3 hours after the fever injection; at least 2 well fevered rabbits were retained for controls.

In the present work $MgCl_2$ was used in 5% solution, amidopyrin in 4% solution. When "combined" the same solutions were used, one immediately following the other; whether injected at the same or different sites was found immaterial.

The results as shown in the curves (Figs. 1 and 2) represent differences in body temperature between treated animals and untreated fever controls, the zero temperature being the temperature at the time (zero hour) of giving the magnesium chlorid.

To produce definite magnesium antipyresis in hay-infusion fever rabbits, about 200 mg.* of the chlorid per kilo are required, as il-

[1] Schütz, J., *Arch. f. exper. Pathol. u. Pharmakol.*, 1916, lxxix, 285.

lustrated in Fig. 1, which shows the effect of 12 injections varying from 150 to 300 mg. per kilo. The difference between the fever control curves and those resulting from 150 mg. per kilo magnesium chlorid represents approximately the maximum variation to be expected with inadequate doses.

It has been shown in this laboratory[2] that 700 mg. per kilo represents approximately the minimal lethal dose of magnesium chlorid for rabbits.

Augmentation of Amidopyrin Antipyresis by Magnesium Chlorid.

Having shown that fevered rabbits respond to doses of magnesium chlorid amounting to less than one-third of the lethal dose, we combined magnesium with another type of antipyretic drug—amidopyrin (pyramidon). Several series of experiments were performed; typical resulting curves appear in Figs. 2 to 4. A comparison of the 3 sets of curves shown in Fig. 2, shows that a very marked antipyretic effect can be elicited by 150 to 200 mg. per kilo of amidopyrin, 100 mg., however, giving a somewhat doubtful effect.

But when to this weak amidopyrin dose is added magnesium chlorid 150 mg. per kilo, these 2 ineffective components produce in combination a profound fall in temperature. The maximum decrease obtained in an average of 9 rabbits was 3.9° F. This is greater than the effect which can be produced separately by either 200 mg. per kilo amidopyrin or 300 mg. per kilo magnesium chlorid. Hence the antipyretic combination exhibits true potentiation or synergism.

With repeated doses, we attempted a closer estimation of the therapeutic equivalent of amidopyrin in terms of its combination with magnesium chloride. In the 4 series of experiments shown in Fig. 3, 5 doses were given each rabbit at half-hourly intervals. In both amidopyrin series, the dosage was 50 mg. per kilo (every half-hour); the averages of 2 sets of 3 rabbits each, showed close agreement (maximum temperature fall about 4° F.). The combination of drugs which was compared with amidopyrin consisted of a mixture of 1 part of amidopyrin with 2 parts of magnesium chlorid. The equivalent of the amidopyrin given alone was found to lie between 50 and 75 mg. per kilo of the combination (Fig. 3).

In a further series, therefore, the 50 mg. amidopyrin dose was compared with 67.5 mg. per kilo of the combination. Injections at half-hourly intervals were performed in the 2 series which each consisted of 3 rabbits. As will be seen from Fig. 4, the combination

[2] Barbour, H. G., and Taylor, W. F., (unpublished experiments).

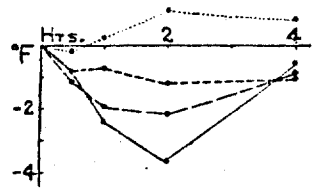


FIG. 1.

Effects of magnesium chlorid given subcutaneously, upon body temperature of fevered rabbits.

Abscissae, time after administration of antipyretic.

Ordinates, temperature changes °F, not absolute but calculated as difference from control fevered rabbits. Five fever controls.

Dosage per kilo: dotted line, 150 mg. (average of 4 rabbits); short dash line, 200 mg. (av. 2 rabbits); long dash line, 250 mg. (av. 4 rabbits); continuous line, 300 mg. (av. 2 rabbits).

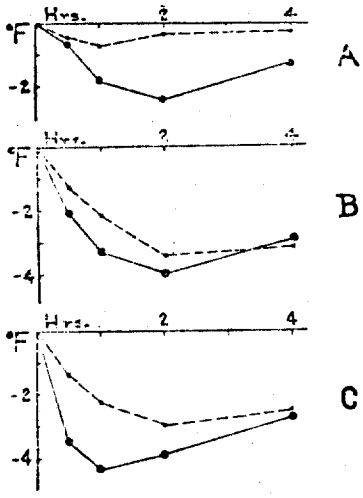


FIG. 2.

Effects of amidopyrin with and without magnesium chlorid, in fevered rabbits.

Dosage per kilo: Continuous lines, effects of amidopyrin 100 mg. plus $MgCl_2$ 150 mg. (A, 2 rabbits; B, 2 rabbits; C, 5 rabbits); broken lines, amidopyrin alone (A, 100 mg. 2 rabbits; B, 150 mg. 2 rabbits; C, 200 mg. 4 rabbits).

A, 2 fever controls; B, 1 fever control; C, 2 fever controls.

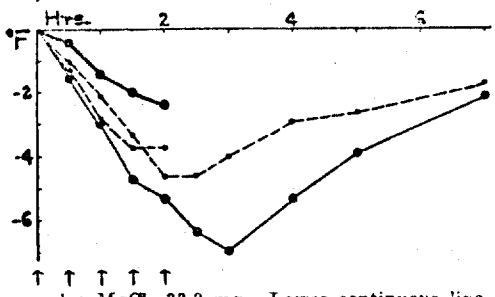


FIG. 3.

Effects of repeated doses of amidopyrin alone and combined with magnesium chlorid, in fevered rabbits.

Temperature changes absolute, no fever controls. Each curve represents the average result from 3 rabbits. Doses given half-hourly shown by arrows.

Dosage per kilo: Upper continuous line, amidopyrin 16.7 mg. plus $MgCl_2$ 33.3 mg. Lower continuous line, amidopyrin 25 mg. plus $MgCl_2$ 50 mg. Both broken lines, amidopyrin alone 50 mg.

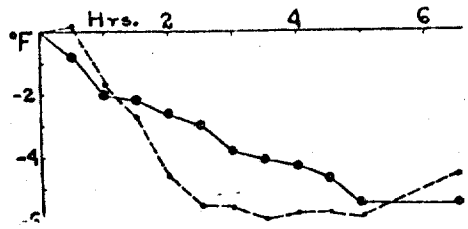


FIG. 4.

Effects of repeated doses of amidopyrin alone and combined with magnesium chlorid, in fevered rabbits.

Temperature changes absolute, no fever controls. Each curve the average from 3 rabbits. Doses given half-hourly shown by arrows.

Dosage per kilo: Continuous

maintained some advantage over amidopyrin for over an hour, that is, until the third dose was beginning to take effect, and an average fall of 2° F. had been attained. From this point on, a decidedly greater temperature fall was seen in the amidopyrin rabbits than in those given the combination.

These results are very significant inasmuch as they indicate for the magnesium-amidopyrin combination a twofold advantage over that dose of amidopyrin, causing an equal fall in 2 hours. (1) This effect was achieved more rapidly by the *combination*. (2) Further temperature reduction, signifying a *transition from the therapeutic into toxic action*, was accomplished more rapidly by the *single drug*. The latter also attained the lowest temperature.

Thus the magnesium combination exhibits both higher therapeutic efficiency and lower toxicity than does amidopyrin alone. The relative toxicity is borne out by the subsequent history of the most heavily injected rabbits (Fig. 4), for a death occurred among the 3 given amidopyrin alone, while all of those given the combination survived.

Relative Toxicity of Magnesium and Amidopyrin Alone and in Combination.

It is frequently found that the toxicity of a combination of 2 drugs differing somewhat in action, is lower than would be predicted from the known action of its components. For example, animals are likely to survive the simultaneous administration of one-half each of the fatal doses of 2 such drugs. This is readily understood from consideration of the fact that the *loci* of toxic action are more extensive. As will be seen from Table I, the minimum lethal doses have been found as follows:

$MgCl_2$	1.05	gm. per kilo.
Amidopyrin	325	mg. per kilo.
Amidopyrin + $MgCl_2$ (1:1 ratio)	500	mg. per kilo.
Amidopyrin + $MgCl_2$ (2:3 ratio)	625	mg. per kilo.

From the first 2 determinations one finds that the predicted minimum lethal doses for the 2 combinations would be 496 and 595 mg. respectively,† from which some antagonism is evident in the case of the 2:3 ratio. Thus in normal mice as well as in fevered rabbits, there is evidence in favor of a decrease in toxicity, as a result of adding to the amidopyrin quantities of magnesium chlorid which increase its efficiency.

† Prediction formula: $ab\ (A+B)/Ab+Ba$ where A parts of a drug having

TABLE I. *Toxicity in Normal White Mice.*

Drug*	Mg. per kilo.	Number of mice	
		Survived	Died
$MgCl_2$	1,000	2	0
	1,050	1	3
	1,100	0	4
	1,200	0	2
	1,300	0	2
	1,400	0	2
	1,500	0	2
	2,000	0	2
Amidopyrin	250	3	0
	300	7	1
	325	1	1
	350	6	4
	375	2	1
	400	7	4
	450	2	5
	500	3	4
Amidopyrin + $MgCl_2$ (2:3)	500	2	0
	562	9	1
	625	9	3
	688	8	3
Amidopyrin + $MgCl_2$ (1:1)	300	1	0
	400	2	0
	450	4	0
	500	8	2
	550	3	1
	600	0	1

*1% solutions.

Summary. 1. Magnesium chlorid proved effectively antipyretic in hay-infusion fever rabbits in doses of 200 mg. per kilo and upwards.

2. By combining doses of magnesium chlorid and amidopyrin, which separately are ineffective, marked antipyresis was produced in such rabbits.

3. The antipyretic efficiency of amidopyrin 100 mg. per kilo plus $MgCl_2$ 150 mg. per kilo was greater than the efficiency of either amidopyrin 200 mg. per kilo or $MgCl_2$ 300 mg. per kilo given separately. (Synergism.)

4. Comparing repeated doses exhibiting equivalent efficiency after 1 or 2 hours, the combination first lowered the temperature more rapidly; nevertheless the amidopyrin later produced a more rapid and extreme (toxic) effect.

5. The amidopyrin-magnesium combination (2:3) proved slightly less toxic to normal white mice than would be predicted from the

037

Conclusion. Combinations of amidopyrin with magnesium chlorid in experimental animals exhibit antipyretic synergism, and, to a lesser extent, diminished toxicity.

MAGNESIUM ABSORPTION IN DOGS AND ITS EFFECT UPON THE METABOLISM OF CALCIUM

HENRY G. BARBOUR AND JAMES E. WINTER

From the Department of Physiology and Pharmacology, School of Medicine, University of Louisville, Kentucky[1]

Received for publication July 7, 1931

In view of the apparent value of magnesium as an adjuvant to certain antipyretics, analgesics and hypnotics (1) and since some of these drugs are likely to be used over long periods, questions relating to the effects of prolonged treatment with magnesium compounds are assuming increasing importance. It is not only desirable to ascertain how much magnesium is absorbed from such compounds and how much is retained, but also to determine what influence this substance has upon the metabolism of calcium.

For some years an impression has prevailed that magnesium tends to displace calcium from the body. The pioneer work in this field was done by Mendel and Benedict (2) who showed from determinations of urine and feces in dogs, that the subcutaneous administration of magnesium salts throws the calcium balance to the negative side. Later Stransky (3) found that magnesium salt injections also reduce the calcium of the blood, and today it seems to be agreed that calcium is displaced from the body when magnesium is introduced subcutaneously.

The feeding of magnesium compounds has, however, led to a different conception. Here the fate of the body calcium seems to depend on the amount of phosphate available in the diet. Malcolm (4) fed $MgCl_2$ thereby slightly increasing calcium excretion in dogs. Hart and Steenbock (5) accomplished the same result in swine, as did Palmer, Eckles and Schutte (6) with cattle. But when the soluble phosphates were increased the calcium loss

[1] In coöperation with the Pharmaceutical Division of the Calco Chemical Company, Bound Brook, New Jersey.

was checked. Furthermore Elmslie and Steenbock (7) found that magnesium feeding in rats has no deleterious effect on the assimilation of calcium; nor did it produce a calcium deficit in bones or retard calcium deposition in healing rickets. Elmslie and Steenbock appear to attribute the lack of deleterious action of magnesium salts in part to the selective absorptive capacity of the digestive tract which, they state, excludes an excess of magnesium.

It seems to us, however, questionable whether the absorptive capacity for magnesium may be regarded as at all constant. Thus substances may be absorbed from a diseased or irritated intestine with untoward results, when given in amounts which would otherwise prove harmless.

But even the form in which magnesium is ingested is likely to determine its rate and total amount of absorption. For example, as we shall now show, magnesium absorption from magnesium gluconate and magnesium lactate can equal that from magnesium oxid fed in equal weight, independent of the magnesium content. The relative amounts of magnesium in these three are approximately 1:2:12.

In our experimental work on dogs soluble organic salts, the lactate and gluconate of magnesium were chiefly used. We present herewith (*a*) the effects on the serum magnesium and calcium of single injections of these salts and of the oxide and (*b*) the effects of prolonged administration of magnesium lactate and gluconate upon the metabolism of magnesium and calcium. Both of these experimental series throw light upon the absorbability of magnesium given in organic salts, and the second series also contributes to the question of magnesium and calcium storage.

I. EXPERIMENTS ON SERUM MAGNESIUM AND CALCIUM

Results of feeding the three magnesium compounds to dogs are illustrated in table 1 which shows the changes produced in both serum calcium and serum magnesium. These dogs had been kept for some time on a diet adequate in calcium, magnesium and phosphate, our Cowgill plan diet 6:

		parts
Casein		120
Sucrose		60
Vitavose		16
Lard		60
Cod liver oil		4
Bone ash		8
Salt mixture		4
	parts	
NaCl	220	
Ca Lactate	88	
Mg Citrate	88	
Ferric Citrate	22	
KI	2	
Total	420)	
		272

Thirteen grams per kilogram were given daily, yielding about 61 calories per kilogram.

In addition to the above diet these dogs had, previous to the blood tests, been receiving daily one of the magnesium compounds given. This accounts for the high level of serum magnesium constantly observed.

In each column of table 1 the first figure is the basal concentration, the second figure the maximum observed after the dose shown. These were the maxima attained in four or five samples taken at regular intervals up to four or five hours after magnesium administration.

The results show a uniform increase in serum magnesium when doses of 200 mgm. per kilogram of oxid, lactate or gluconate were given. Smaller doses were less effective; 50 mgm. gluconate per kilogram did not raise the serum magnesium level, while 100 mgm. MgO per kilogram gave relatively slight increase. The calcium content of the serum was reduced in only one instance in seventeen experiments done on 5 dogs, thus indicating that a single feeding of considerable magnesium does not as a rule displace calcium from the blood. The table also suggests that the three compounds used were nearly equivalent gram for gram in their capacity to increase the serum magnesium. This occurred in spite of the fact that the gluconate contains only one-twelfth as

much magnesium and the lactate one-seventh as much magnesium as does the oxid.

II. EXPERIMENTS ON THE CALCIUM AND MAGNESIUM METABOLISM

We present now the results of four metabolism experiments upon three healthy, adult, female dogs. In one pair of these experiments the basal diet was adequate in magnesium, calcium and phosphorus (diet 6, page 609). In the other pair of experi-

TABLE 1

Maximum changes in serum Ca and Mg after oral administration of MgO, Mg gluconate or Mg lactate

AMOUNT OF DOSE	ORAL DOSE OF MgO		ORAL DOSE OF Mg GLUCONATE		ORAL DOSE OF Mg LACTATE	
	Ca	Mg	Ca	Mg	Ca	Mg
mgm. per kgm.	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
50			10.9–12.0	4.0–4.0		
			10.6–10.8	4.0–4.0		
			10.1–10.8	4.4–4.4		
100	11.0–11.8	3.6–3.7				
	10.3– 9.5	3.8–3.9				
	10.6–10.9	4.0–4.5				
200	10.1–11.0	4.6–5.2	10.7–11.4	4.4–5.5	10.2–12.8	4.2–5.2
	10.8–12.3	4.3–5.4	10.1–11.1	4.2–5.4	11.0–12.5	4.6–5.2
	10.8–11.4*	4.8–6.1*	9.1–10.4	4.4–5.0	10.2–10.8	4.1–5.5
			10.6–10.5*	3.8–4.0*	10.4–10.6*	3.7–4.0*

* Two hours only.

ments the basal diet was considerably lower in all these constituents. Otherwise this diet, 6A, was of the same composition as diet 6. The differences consisted in the substitution of 0.625 gram calcium phosphate for each gram of bone ash, and the omission of magnesium citrate from the salt mixture. One animal of each pair was given magnesium lactate, the other magnesium gluconate, during the experimental periods proper. The magnesium compounds were given in aqueous solution, by stomach tube before each day's meal.

All the animals were easily catheterized and the urine and feces

were collected and analyzed in periods varying in length from four to seven days. The period of magnesium administration varied in the four experiments from nineteen to sixty-three days. The feces of the different periods were separated by carmine or charcoal feeding. The animals continued in an excellent state of nutrition throughout these experiments, consuming all of their standard ration and remaining free from diarrhea.

TABLE 2

Dog 126. Effects of magnesium lactate on magnesium metabolism

DATES	DAILY INTAKE	DAILY OUTPUT			
	Total MgO	Urine MgO	Feces MgO	Total MgO	Balance MgO
	gram	*gram*	*gram*	*gram*	*gram*
January 22–27	0.165	0.026	0.193	0.219	−0.054
January 27–February 1		0.009	0.484	0.493	−0.328
February 1–6	0.555	0.004	0.479	0.483	+0.072
February 6–10		0.020	0.650	0.670	−0.115
February 10–17		0.006	0.283	0.289	+0.266
February 17–24		0.030	0.337	0.367	+0.188
February 24–March 3		0.013	0.124	0.137	+0.418
March 3–9		0.017	0.321	0.338	+0.217
March 9–15		0.036	0.288	0.324	+0.231
March 15–22		0.010	0.298	0.308	+0.247
March 22–29		0.007	0.207	0.214	+0.341
March 29–April 5		0.003	0.205	0.208	+0.347
April 5–12	0.165	0.030	0.252	0.282	−0.117
April 12–17		0.006	0.292	0.298	−0.133

* Per kilogram 0.2 gram magnesium lactate daily.
Average daily MgO absorbed, 0.26 gram.
Average daily MgO retained, 0.24 gram.

Effects of magnesium lactate during a diet adequate in magnesium, calcium and phosphorus

Dog 126 weighing 12.7 kgm. was placed on diet 6 from January 22 to April 17, 1930, receiving 13 grams per kilogram daily. From February 1 to April 5 inclusive, she was given daily per kilogram 0.2 gram magnesium lactate. The effects of the magnesium lactate during these sixty-four days, on the magnesium metabolism

are shown in table 2, on calcium metabolism in table 3. Two fore-periods and two after-periods are also shown. A considerable retention of both magnesium and calcium may be noted during lactate administration although the dog presented a negative balance as regards both magnesium and calcium before the lactate was started.

TABLE 3

Dog 126. Effects of magnesium lactate on calcium metabolism

DATES	DAILY INTAKE	DAILY OUTPUT			
	Total CaO	Urine CaO	Feces CaO	Total CaO	Balance CaO
	grams	*gram*	*grams*	*grams*	*grams*
January 22–27		0.030	2.131	2.161	+1.155
January 27–February 1		0.034	4.601	4.635	−1.319
February 1–6	3.316	0.039	2.702	2.741	+0.575
February 6–10		0.052	4.968	5.020	−1.704
February 10–17		0.099	2.710	2.809	+0.507
February 17–24		0.015	2.071	2.086	+1.230
February 24–March 3		0.014	2.423	2.437	+0.879
March 3–9		0.011	2.006	2.017	+1.299
March 9–15		0.032	1.823	1.855	+1.461
March 15–22		0.011	2.001	2.012	+1.304
March 22–29		0.025	1.481	1.506	+1.860
March 29–April 5		0.046	1.294	1.310	+2.006
April 5–12		0.006	1.636	1.642	+1.674
April 12–17		0.018	2.991	3.009	+0.307

* Per kilogram 0.2 gram magnesium lactate daily.
Average daily CaO retained, 0.84 gram.

While the level of the urinary magnesium was not definitely altered by the lactate, it is evident that there was no marked increase in the magnesium oxid of the feces. By comparison of the latter data with the intake it is found that the daily net absorption of magnesium oxid amounted to 0.26 gram, practically all of which was stored by the body.

The urinary calcium showed, during the first seventeen lactate days, a progressive increase but this was followed by a period of forty days in which the calcium excretion by this route, was below

normal. The net result of the entire period was an average daily CaO retention of 0.84 gram. This dog, therefore, affords decided evidence that magnesium administration may promote the storage of both magnesium and calcium in the body.

TABLE 4

Dog 91. Effects of magnesium gluconate on magnesium metabolism

DATES	DAILY INTAKE	DAILY OUTPUT			
	Total MgO	Urine MgO	Feces MgO	Total MgO	Balance MgO
	gram	*gram*	*gram*	*gram*	*gram*
January 22–27	0.367	0.007	0.315	0.322	+0.045
January 27–February 1		0.007	0.337	0.344	+0.023
February 1–5		0.009	0.229	0.238	+0.129
February 5–10		0.009	0.103	0.112	+0.255

Per kilogram 0.2 gram magnesium gluconate daily.
Average daily MgO absorbed, 0.12 gram.
Average daily MgO retained 0.11 gram.

TABLE 5

Dog 91. Effects of magnesium gluconate on calcium metabolism

DATES	DAILY INTAKE	DAILY OUTPUT			
	Total CaO	Urine CaO	Feces CaO	Total CaO	Balance CaO
	grams	*gram*	*grams*	*grams*	*grams*
January 22–27	3.588	0.056	4.292	4.348	−0.760
January 27–February 1		0.026	4.074	4.100	−0.512
February 1–5		0.026	2.153	2.175	+1.413
February 5–10		0.022	0.600	0.622	+2.966

Per kilogram 0.2 gram magnesium gluconate daily.
Average daily CaO retained, 0.74 gram.

Effects of magnesium gluconate during a diet adequate in magnesium, calcium and phosphorus

The next dog, no. 91, weighed 11.9 kgm. and was kept on diet 6 from January 6 to February 10, receiving 15 grams per kilogram per day. From January 10 to 18, 50 mgm. magnesium gluconate per kilogram was given and for the next four days daily amounts

averaging 150 mgm. per kilogram. The magnesium and calcium analyses given respectively in tables 4 and 5, cover the final nineteen days of the diet, during which the dog received daily magnesium gluconate 200 mgm. per kilogram.

The magnesium balance was maintained throughout this time, and during the last nine days magnesium was well absorbed and retained. The net average daily absorption, during the entire time the dog received per kilogram 200 mgm. gluconate daily, was 0.12 gram MgO, retention 0.11 gram MgO. While the

TABLE 6

Dog 132. Effects of magnesium lactate on magnesium metabolism

DATES	DAILY INTAKE	DAILY OUTPUT			
	Total MgO	Urine MgO	Feces MgO	Total MgO	Balance MgO
	gram	*gram*	*gram*	*gram*	*grams*
May 17–21	0.117	0.026	0.351	0.377	−0.260
May 21–26		0.025	0.312	0.337	−0.220
* May 26–June 2	1.250	0.006	0.565	0.571	+0.680
June 2–9		0.022	0.819	0.841	+0.410
June 9–16		0.033	0.290	0.323	+0.930
June 16–23		0.033	0.156	0.189	+1.060
June 23–30		0.013	0.395	0.409	+0.840
June 30–July 7	0.117	0.012	0.297	0.309	−0.190

* Per kilogram 0.4 gram magnesium lactate daily.
Average daily MgO absorbed, 0.80 gram.
Average daily MgO retained, 0.78 gram.

urinary calcium was somewhat high and the calcium balance negative at the start, it will be observed that there was a marked storage or calcium during the last two periods giving a net retention for the period averaging 0.74 gram CaO daily.

Effects of magnesium lactate during a diet low in magnesium, calcium and phosphorus

The basal diet of the next two experiments was 6A. Dog 132 (18.1 kgm. from May 17 to July 7, received 13 grams per kilogram per day of this diet. This afforded an intake of both calcium and

phosphorus about 25 per cent lower than diet 6, while the magnesium was reduced by nearly 50 per cent.

Under these conditions, a thirty-four-day period of magnesium lactate administration caused such an increase in absorption and retention of MgO (table 6) that magnesium was stored at a rate about twice as rapid as with the previous lactate dog. The average daily MgO absorption was 0.80 gram, retention 0.78 gram.

The calcium metabolism of this dog (table 7) was affected in just the opposite way from that of the first dog under magnesium

TABLE 7

Dog 132. Effects of magnesium lactate on calcium metabolism

DATES	DAILY INTAKE	DAILY OUTPUT			
	Total CaO	Urine CaO	Feces CaO	Total CaO	Balance CaO
	grams	*gram*	*grams*	*grams*	*grams*
May 17–21		0.046	7.10	7.146	+3.80
May 21–26		0.040	1.97	2.010	−1.34
May 26–June 2		0.049	3.20	3.250	−0.10
June 2–9		0.036	4.90	4.940	−1.59
* June 9–16	3.35	0.090	4.40	4.490	−1.140
June 16–23		0.033	5.70	5.730	−2.38
June 23–30		0.011	1.90	1.910	−1.44
June 30–July 7		0.053	3.12	3.170	−0.18

* Per kilogram 0.4 gram magnesium lactate daily.
Average daily CaO lost, 1.33 grams.

lactate. An average daily net loss of 1.33 grams CaO was observed. While the better magnesium retention is probably related to the lower basal magnesium intake of this dog, the failure to store calcium may be attributed to a lack of sufficient phosphate.

Effects of magnesium gluconate during a diet low in magnesium, calcium and phosphorus

Dog 126 which had originally been given magnesium lactate after four weeks rest on a mixed diet, weighed 16.1 kgm. She was then placed on diet 6A from April 13 to July 7, receiving

TABLE 8

Dog 126. Effects of magnesium gluconate on magnesium metabolism

DATES	DAILY INTAKE	DAILY OUTPUT			
	Total MgO	Urine MgO	Feces MgO	Total MgO	Balance MgO
	gram	*gram*	*gram*	*gram*	*gram*
May 13–17		0.036			
May 17–21	0.104	0.049	0.134	0.183	−0.079
May 21–26		0.022	0.079	0.101	+0.003
May 26–June 2		0.019	0.341	0.431	+0.081
June 2–9		0.034	0.417	0.451	+0.061
* June 9–16	0.512	0.038	0.185	0.223	+0.289
June 16–23		0.040	0.254	0.294	+0.218
June 23–30		0.022	0.337	0.359	+0.153
June 30–July 7	0.104	0.031	0.141	0.172	−0.068

* Per kilogram 0.4 gram magnesium gluconate daily.
Average daily MgO absorbed, 0.21 gram.
Average daily MgO retained, 0.16 gram.

TABLE 9

Dog 126. Effects of magnesium gluconate on calcium metabolism

DATES	DAILY INTAKE	DAILY OUTPUT			
	Total CaO	Urine CaO	Feces CaO	Total CaO	Balance CaO
	grams	*gram*	*grams*	*grams*	*grams*
May 13–17		0.047			
May 17–21		0.055	3.30	3.355	−0.39
May 21–26		0.011	1.91	1.921	+1.05
May 26–June 2		0.017	3.10	3.117	−0.15
June 2–9		0.018	3.40	3.418	−0.45
* June 9–16	2.97	0.103	3.90	4.003	−1.03
June 16–23		0.084	6.10	6.184	−3.21
June 23–30		0.101	3.70	3.801	−0.83
June 30–July 7		0.035	4.10	4.135	−1.17

* Per kilogram 0.4 gram magnesium gluconate daily.
Average daily CaO lost, 1.13 gram.

13 grams per kilogram daily. During a thirty-four-day period beginning April 26, she was given daily per kilogram 0.4 gram magnesium gluconate. The results on the magnesium and calcium metabolism are shown in tables 8 and 9 respectively. While the urinary magnesium was not definitely altered and the loss of magnesium by the feces was increased, the magnesium balance was definitely positive throughout the entire administration. The average daily MgO absorption was 0.21 gram, retention 0.16 gram. The urinary calcium appeared to be increased during the latter half of the gluconate period and the calcium balance was decidedly decreased, the average daily calcium loss amounting to 1.13 grams. This decrease in the stored calcium of the body must again be attributed to the low phosphate intake.

DISCUSSION

Magnesium absorption

While the magnesium ion is not as well absorbed from the alimentary tract as certain others, especially the monovalent ions, the results of this paper have added considerably to our impression that the amount of magnesium absorbed from non-purgative doses of its salts is commonly underestimated. Evidence for this view includes increased efficacy of analgesics and hypnotics given with magnesium to which reference was made in our introduction.

In the next place comes the evidence of increased serum magnesium. Winter and Richey (9) in this laboratory, found that magnesium chlorid in dogs caused an average gain of 1 mgm. per cent or more in the serum magnesium. Later the same authors (10) found that the magnesium serum level in man could occasionally be raised 0.5 mgm. per cent or more after small doses of magnesium chlorid or magnesium oxid, especially if given with ginger, alcohol or aspirin. In the first table of the present paper, we have again shown increases of about 1 mgm. per cent in the serum magnesium level of dogs after doses of 200 mgm. per kilogram of magnesium oxid; and similar changes resulted from amounts of the gluconate and lactate containing far less magnesium.

Now there is available evidence of magnesium absorption from

the analyses of intake and excreta both in dogs, in the present paper, as well as in the subsequent work of Carswell and Winter (8) in man. Absorption increase does not appear from the urine analyses alone, since the kidneys were apparently excreting their maximum for the conditions before the extra magnesium was added. However, the net absorption is striking when calculated from the difference between intake MgO and the MgO of the feces.

For over two months, one dog absorbed 45 per cent of magnesium administered chiefly in the form of lactate, and during another period of over one month, absorbed 41 per cent of magnesium given mostly as gluconate. Another dog on gluconate for a nineteen day period absorbed 33 per cent of the magnesium

TABLE 10

Relation of magnesium and calcium retention to intake of calcium, magnesium and phosphate

DOG NUMBER	EXTRA MAGNESIUM GIVEN AS	DAYS	MgO NET ABSORBED	PER KILOGRAM PER DAY				
				MgO		CaO		P_2O_5 Intake
				Intake	Retention	Intake	Retention	
			per cent	*millimols*	*millimols*	*millimols*	*millimols*	*millimols*
126	Lactate	63	45	1.1	+0.47	4.5	+1.2	4.2
91	Gluconate	19	33	0.7	+0.23	5.4	+1.1	4.9
132	Lactate	34	64	1.7	+1.08	3.3	−1.3	3.5
126	Gluconate	34	41	0.8	+0.25	3.3	−1.2	3.5

given. A third dog given magnesium lactate for thirty-five successive days, absorbed her magnesium intake to the extent of 64 per cent. From these figures it appears that magnesium absorption from the lactate was better than from the gluconate (see table 10).

Of especial interest is the comparison of the above magnesium absorption with that found by Carswell and Winter on themselves. They found with magnesium lactate that 0.1 gram per kilogram (which corresponds to one-half of our smaller dog dose) was about all that could be tolerated daily without uncomfortable laxative action. While taking this amount they absorbed respectively 42 and 61 per cent of their total magnesium intake, which figures agree closely with our dog results.

We may, therefore, conclude not only that inorganic and organic salts of magnesium markedly raise the level of serum magnesium, but also that from large amounts of organic magnesium salts given orally, from one- to two-thirds may readily be absorbed.

Magnesium and calcium metabolism

In order to compare the effects of magnesium feeding in the four experimental series, the intake of magnesium, calcium and phosphate and the retention of the first two during the magnesium feeding have all been calculated as millimols per kilogram per day, and are thus presented in table 10. The magnesium intake shown includes both the basal and the added magnesium. The table shows that magnesium storage caused by the lactate administration, may be doubled when the basal diet is low in magnesium; this does not however seem to be true of the gluconate.

Retention of calcium, as already pointed out, took place in the presence of adequate phosphorus. A calcium deficit, such as results from subcutaneous injections of magnesium salt, appeared however when the phosphate intake was lowered. Our results not only accord with the conclusions of Steenbock and others that adequate phosphate is essential to preserve the calcium level, but they suggest further that magnesium plus adequate phosphate may even serve to build up the body reserve of both magnesium and calcium. Further support for this hypothesis has arisen out of the work of Carswell and Winter.

CONCLUSIONS

1. Large amounts of magnesium were absorbed from the alimentary tract of dogs by the use of soluble organic compounds.

2. Prolonged administration of large doses of magnesium lactate or gluconate to dogs, was accompanied by no loss of calcium when the phosphate intake was adequate. When the P_2O_5 intake exceeded 4 millimols per kilogram per day, extra calcium was retained.

Analytical methods: Serum calcium, Clark and Collip; serum magnesium, Denis; calcium and magnesium of food, urine and

feces, McCrudden, gravimetric; phosphate, uranium acetate. Analyst: James H. Warner.

REFERENCES

(1) Winter, J. E., Richey, C. H., and Barbour, H. G.: Jour. Pharmacol. and Exper. Therap., 1930, xxxviii, 343.
Barbour, H. G., and Taylor, W. F.: Jour. Pharmacol. and Exper. Therap., 1931 (in press).

(2) Mendel, L. B., and Benedict, S. R.: Amer. Jour. Physiol., 1909, xxv, 1.

(3) Stransky, E.: Arch. exper. Pathol. u. Pharmakol., 1925, lxxviii, 122.

(4) Malcolm, J.: Jour. Physiol., 1905, xxxii, 183.

(5) Hart, E. B., and Steenbock, H.: Jour. Biol. Chem., 1913, xiv, 75.

(6) Palmer, L. S., Eckles, C. H., and Schutte, D. J.: Proc. Soc. Exper. Biol. and Med., 1928, xxvi, 58.

(7) Elmslie, W. P., and Steenbock, H.: Jour. Biol. Chem., 1929, lxxxii, 611.

(8) Carswell, H. E., and Winter, J. E.: Jour. Biol. Chem., 1931, xciii, 411.

(9) Winter, J. E., and Richey, C. H.: Jour. Pharmacol. and Exper. Therap., 1931, xli, 245.

(10) Winter, J. E., and Richey, C. H.: Jour. Pharmacol. and Exper. Therap., 1931, xlii, (in press).

J. Pharmacol + Exp. Therap., 42:321-331, 1931

THE INFLUENCE OF MAGNESIUM CHLORID UPON THE NARCOTIC AND TOXIC EFFECTS OF SODIUM BARBITAL

H. G. BARBOUR AND W. F. TAYLOR[1]

From the Department of Physiology and Pharmacology, School of Medicine, University of Louisville, Kentucky

Received for publication February 7, 1931

Barbital, perhaps most widely used of all hypnotic drugs at the present time, presents the drawback of very persistent after-effects in a large percentage of cases. Magnesium, on the other hand, while an effective depressant of the entire nervous system with relatively brief action, offers a therapeutic range of dosage so limited as to preclude its narcotic use alone.

Issekutz (1) was, therefore, led to recommend that magnesium sulfate when used in war tetanus, be combined with a relatively high dose of some other hypnotic drug This advice was supported by studies of combinations of magnesium sulfate with a variety of methane and alkaloidal narcotics. The report of Issekutz does not refer to barbital but he obtained the following results with pheno-barbital.

For magnesium sulfate the narcotic threshold was 1.25 grams per kilogram and the lethal dose 1.5. He therefore called the "narcotic range" 0.25 grams per kilogram, or 20 per cent of the narcotic dose. Against the background of pheno-barbital, however, in dosage of 60 mgm. per kilogram, narcosis could be obtained with as low as 800 mgm. of $MgSO_4$, which did not become fatal until increased to 1.4. In this case the "narcotic range" of $MgSO_4$ became 0.6 gram or 75 per cent of the narcotic dose. When the pheno-barbital was increased to 80 mgm. per kilogram the "relative narcotic range" of $MgSO_4$ became 116.6

[1] In coöperation with the Pharmaceutical Division of the Calco Chemical Company, Bound Brook, New Jersey.

per cent. This application of the conceptiom of narcotic range, it will be noted takes account only of one of the component drugs; since only one drug at a time was varied in amount we do not learn the therapeutic range of any particular ratio of combination.

In spite of the impracticability of using magnesium salts by themselves, it seems reasonable to attempt taking advantage of their hypnotic properties by combining them in suitable proportions with the safer methane derivatives.

We were especially encouraged to undertake such studies by the reports of Loewe's (2) work upon combinations of hypnotics (especially barbital) with antipyretics, as well as by the results in our own laboratory in the field of antipyresis. Loewe and his co-workers showed, for example, that such combinations as barbital and phenacetin (3) or barbital and acetyl salicylic acid (4) exhibited lower toxicity than would be predicted from the toxicities of the individual components. This apparently "protective antagonism,"[2] if not cellular, may depend upon such factors as diversity in the respective anatomical loci of toxicity as well as in the respective times of peak action. Several antipyretic drugs have been tested by us in combination with magnesium, and such "protective antagonism" was indicated in the cases of amidopyrin (5), salicylate (6), phenyl cinchoninic acid (7) and of phenacetin (8). As regards therapeutic action, however, in the antipyretic group, the results as a rule have pointed rather to "potentiation."

The following work was undertaken to throw light upon the relations obtaining between magnesium and barbital variously combined, both as regards toxicity and hypnotic action. The work was done entirely with magnesium chlorid and sodium barbital. These were given by subcutaneous injection to normal, healthy, adult rabbits, and in some of the toxicity experiments, to mice. All mention of dosage which follows refers to milligrams per kilogram animal.

[2] We use the terms "antagonism" and "potentiation" simply to indicate whether the dose of a two-drug combination necessary to produce a given effect is larger or smaller than the dose "predicted" by the formula: $ab(A + B)/Ab + Ba$ where A parts of a drug having M.L.D. $= a$ are combined with B parts of a drug having M.L.D. $= b$.

TOXICITY

In seeking a ratio which will best exhibit protective antagonism, one is inclined to combine two drugs in proportion to their respective fatal doses. In this way, whatever dose of the combination is used should theoretically derive its toxicity in equal measure from the two components. Loewe has in a general way confirmed this viewpoint.

Our first toxicity experiments were on mice and included tests with two different ratios. Barbour and Winter had previously found the M.L.D. of magnesium chlorid for mice to be about 1050 mgm. per kilogram, while in the present work, sodium barbital tests on twenty-six mice indicated a M.L.D. of 475 mgm. Eight mice were used to test a combination in the proportion of one part of sodium barbital to four parts of $MgCl_2$, with the result that a M.L.D. of 844 mgm. was indicated. This agrees with the "predicted" dose (845 mgm.) for this ratio. When, however, a 1:2 combination was used, corresponding practically to the ratio between the respective lethal doses, some degree of "protective antagonism" was found. Tests on fourteen mice indicated a M.L.D. of 788 mgm. as against 748 which would be "predicted" for this ratio.

The toxicity of the two drugs separately and combined in rabbits, is set forth in table 1. Roemer (9) and others have established 400 mgm. per kilogram as the M.L.D. of sodium barbital for rabbits. Inasmuch as Roemer found that all rabbits tested survived 350 mgm. while 100 per cent succumbed to 400 mgm., one is clearly safe in assuming that the M.L.D. does not exceed 400 mgm. according to the criterion of Trevan (10) (the dose which just kills 50 per cent of all animals). For $MgCl_2$ we found a 60 per cent fatality among ten rabbits given 725 mgm. which we have, therefore, taken as the probable minimal lethal dose. The combination given in various doses of the 2:1 ratio, caused no fatalities until 220 sodium barbital plus 440 $MgCl_2$ was given. The table shows that this, or possibly a higher dose, may be taken as the M.L.D. of the 1:2 combination. In figure 1, a chart of the type devised by Loewe for showing dose relationships,

curve *C* is plotted from the three points which indicate the respective M.L.D.'s just mentioned. Contrasted with this curve is seen the so-called "addition line," *B*, which is a straight line connecting the found M.L.D.'s of the two separate drugs, and, therefore, includes for any given ratio the dose giving simple summation of effect as opposed to either synergism or antagonism. In other words, line *B* represents the predicted M.L.D.'s for all ratios. Since the end points taken for this summation line are both probably a little higher than the true M.L.D.'s of the respective drugs,

TABLE 1

Toxicity for rabbits

Na BARBITAL		$MgCl_2$	SURVIVED	DIED	FATALITY
mgm. per kgm.		*mgm. per kgm.*			*per cent*
350 400					0 100 } Roemer (9)
		700	11	1	9
		725	10	6	60
		750	2	3	60
		775	0	2	100
188	+	376	2	0	0
192	+	385			Predicted m.l.d.
196	+	392	2	0	0
212	+	424	4	0	0
220	+	440	2	2	50
230	+	460	1	1	50
240	+	480	3	1	25

and since the M.L.D. for the 1:2 combination is plotted at a conservatively low point, we may safely say that in rabbits this combination gives a "protective antagonism" at least as great as the 14 per cent increase shown over the predicted M.L.D.

NARCOSIS

Definitions. "Light narcosis" in this work is taken as beginning coincidently with the first appearance of any muscular incoordination; "deep narcosis" begins with the loss of the head-righting reflex; "partial recovery" starts at the appearance of any

p. 325

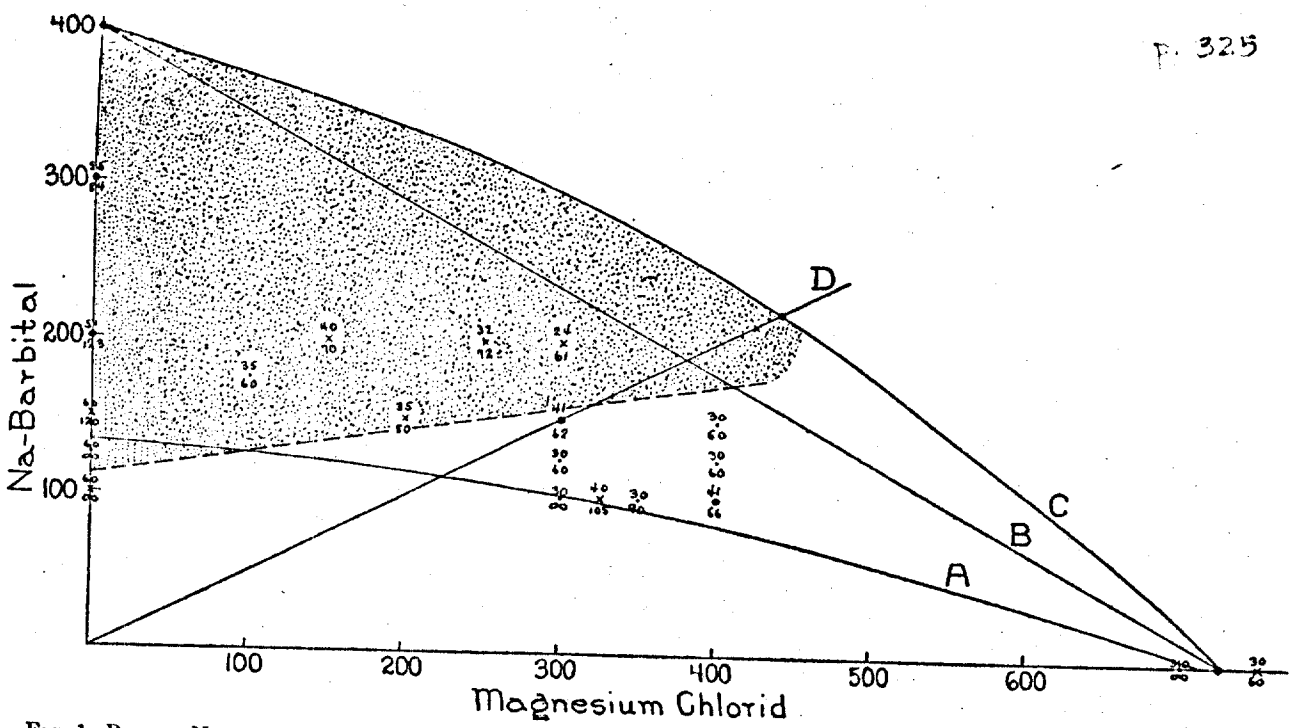


FIG. 1. RABBIT NARCOTIC AND TOXIC DOSAGE OF SODIUM BARBITAL, MAGNESIUM CHLORID AND THEIR COMBINATIONS

Ordinates: Sodium barbital milligrams per kilogram; abscissae: magnesium chlorid milligrams per kilogram. *A*, threshold dosage for deep narcosis; *B*, toxicity line (predicted or additive); *C*, toxicity line based on findings with Na-barbital plus $MgCl_2$ in 1:2 ratio; small dots, results from individual rabbits; crosses, average result from two rabbits; large dots, average results well established by numerous experiments (see tables 1 and 2). Line *D* represents all sub-lethal doses of a combination of one part sodium barbital to two parts of magnesium chlorid. Small figures above points indicate minutes required to produce light narcosis; figures below points, minutes required to produce deep narcosis; (infinity = no deep narcosis). Long dash line, threshold dose for complete recovery within ten hours (shaded area longer persistence of action).

sign indicating a return in the direction of normal function, while "complete recovery" corresponds to the disappearance of the last sign of incoördination.

Sodium barbital. In twenty-eight experiments in which this drug alone was given in doses of 100 to 300 mgm., fifty to sixty minutes were required to produce light narcosis. The threshold for deep narcosis lay between 125 and 150 mgm. But this stage required at least two hours to appear with doses of 200 mgm. and under. The effects of 200 and 300 mgm. doses upon series of twelve and ten animals respectively are shown in table 2. Since partial and complete recovery from barbital occurred more often

TABLE 2

Na-barbital narcosis with and without $MgCl_2$

NUMBER OF EXPERIMENTS	AMOUNT INJECTED		M.L.D.	AVERAGE TIME REQUIRED TO PRODUCE			
	Barbital	$MgCl_2$		Light narcosis	Deep narcosis	Partial recovery	Complete recovery
	mgm. per kgm.	*mgm. per kgm.*	*per cent*	*minutes*	*minutes*	*minutes*	*minutes*
12	200	0	50	51 ±2.9	123 ±22.1	>467	>600
10	300	0	75	55 ±2.6	84 ± 3.8	>696	>810
12	150	300	68	41 ±1.9	62 ± 2.0	233†	560†
7	100	400	81*	41 ±4.5	66 ± 2.7	281	475

* Of predicted.

† Eight rabbits only.

during the night following the early morning injections, and the exact time was not observed in most cases, the barbital data under these heads in table 2, show merely the average length of time that the animals were observed to be still completely or partially depressed.

In a few tests with smaller doses, less than ten hours were required to recover from 100 mgm. of barbital while animals given 125 or any higher dose almost never recovered completely within that period. Hence in figure 1 the ten-hour recovery line (dashes) meets the barbital ordinate just above the 100 mgm. point.

A glance along the ordinate of figure 1 summarizes the effects of various doses of barbital. Four hundred milligrams per kilo-

gram, M.L.D.; 300 mgm., light narcosis in fifty-five minutes, deep in eighty-four minutes; 200 mgm., light narcosis in fifty-one minutes, deep in one hundred twenty three minutes; 150 mgm., light narcosis in sixty minutes, deep in one hundred twenty minutes; 100 to 125 mgm., light narcosis in sixty minutes, no deep narcosis. Line A for deep narcosis threshold, just over 125 mgm.; line (dashes) for ten-hour recovery, between 100 and 125 mgm.

Magnesium chlorid. Any effective subcutaneous dose of magnesium chlorid will produce light narcosis in about thirty minutes. Some of the toxicity experiments with this salt were kindly made by Dr. Winter, and some by Mr. Warner. Of eleven rabbits given 700 mgm. per kilogram, only five showed light narcosis and one deep narcosis with death. Of ten rabbits given 725 mgm. all showed deep narcosis and six died, while of five rabbits given 750 mgm. four showed deep narcosis and three died.

Therefore, the abscissae of figure 1 which represents $MgCl_2$ dosage, meets the threshold line for deep narcosis between 700 and 725 mgm., which latter figure is taken as the M.L.D. for rabbits. Thus is confirmed the finding that $MgCl_2$ has a very limited therapeutic range.

Sodium barbital plus magnesium chlorid. A variety of combinations has been employed in our work but chief attention (as with toxicity) was given to the 1:2 ratio. One should compare in table 2, the results of injecting 68 per cent of the observed M.L.D. of this combination with those following sodium barbital injected in 75 per cent of its M.L.D. For the onset of either light or deep narcosis in ten experiments with sodium barbital, about one-third longer was required than in twelve experiments with the 1:2 combination. The small standard deviations of the average results should carry conviction, even with this relatively small series of experiments, that this combination induces narcosis more quickly, even with a smaller proportion of the lethal dose, than does barbital alone.

Recovery as well as narcosis began much slower with the 1:2 combination than with sodium barbital alone. Partial recovery apparently required about one-third as long and complete re-

covery about two-thirds as long for the 1:2 combination as for the sodium barbital.

A specific experiment will now be cited in illustration of the fact that a dose of the 1:2 combination causes a quicker narcosis and very much quicker recovery than a somewhat more toxic dose of Na-barbital alone.

December 10, 1927,	Rabbit 93	Rabbit 93
Color	Gray	White
Weight, grams	2,062	2,600
Na-barbital, grams per kilogram	300	150
$MgCl_2$ grams per kilogram	0	300
Total dosage represents of minimal lethal dose, per cent	75	68
Time of injection	7:30 a.m.	7:28 a.m.
Slight narcosis appeared in barbital plus $MgCl_2$ rabbit		8:10
Photo no. 1 taken (see fig. 2)	8:15	8:15
Slight narcosis appeared in barbital rabbit	8:30	
Deep narcosis began	9:00	8:25
Recovery began (raises head) in barbital plus $MgCl_2$ rabbit		10:50
Photo no. 2 taken (shows barbital plus $MgCl_2$ rabbit nearly recovered	1:00 p.m.	1:00 p.m.
Photo no. 3 taken (shows barbital plus $MgCl_2$ rabbit recovered; barbital rabbit still in narcosis)	4:40	4:40

The rabbit receiving the combination is the white one in figure 2, shown in each case on the right; the rabbit on the left received a slightly more toxic dose of Na-barbital alone. The animals were injected practically simultaneously. The first picture shows the combination rabbit well narcotized fifteen minutes before narcosis began in the Na-barbital rabbit. This was forty-five minutes after the injections. The second picture taken five and one-half hours after the injections, shows the combination rabbit nearly recovered, the Na-barbital rabbit being still in deep narcosis. Nine hours and ten minutes after the injections, the Na-barbital rabbit was still in deep narcosis, as shown by the third picture.

The phenomenon of accelerated onset and accelerated recovery from barbital narcosis just illustrated, occurs over a wide range of dosage and of combination ratios. In figure 1, these different ratios could be indicated by diagonals from the axis similar to the

line *D*. At various points in the diagram are indicated the various narcosis results. A light dot indicates a result from a single

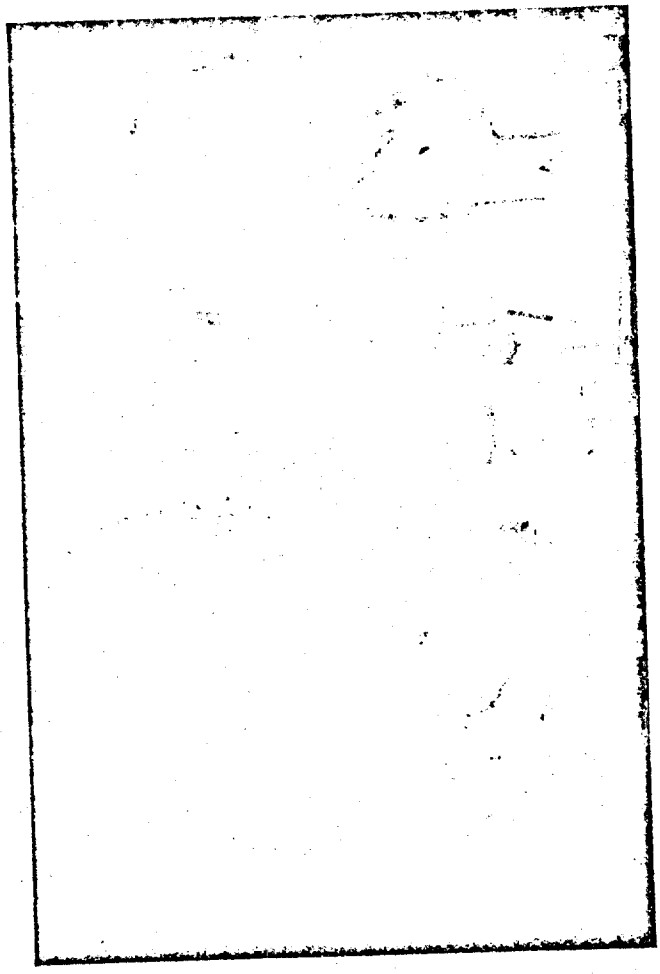


FIG. 2. RABBITS INJECTED WITH SODIUM BARBITAL (LEFT) AND SODIUM BARBITAL PLUS MAGNESIUM (RIGHT)

The combination gives more rapid onset and more rapid recovery (details in text).

rabbit, a cross an average from two rabbits, while the heavy dots are well established by the number of experiments indicated in tables 1 or 2. Above each point is given the time in minutes re-

quired for light narcosis and below for deep narcosis; a general relationship to dosage is evident. The point of incidence of the threshold line (A) for deep narcosis with the ordinate for 100 mgm. sodium barbital, is determined by a series of experiments in which this dose of barbital was combined with varying amounts of $MgCl_2$. When such a combination included 325 mgm. of $MgCl_2$, one hundred five minutes were required for deep narcosis, but when the $MgCl_2$ moiety was reduced to 300 mgm., narcosis never became deep. Thus the line A passes between these two points, determining its approximate course.

This develops the diagram sufficiently to see how the "therapeutic range" increases the further one gets from pure $MgCl_2$. This range is determined by the distance between the lines A and C. When the 2:1 combination (diagonal D) is reached the "relative narcotic range" in the sense of Issekutz, is evidently increased to well over 100 per cent. In about this region, however, one meets the area which has been shaded to represent the disadvantageous feature of sodium barbital, namely, persistent after-effect. This area is bounded by the ten-hour recovery line of which the upward slope indicates that with increasing doses of $MgCl_2$ the threshold dose for barbital action is raised. Perhaps the dosage represented by the intersection of lines A and D would afford an optimum hypnotic injection for a rabbit where the human desiderata are in view. One sees in this region relatively rapid onset of light and deep narcosis and complete recovery within ten hours, by the employment of a relatively small fraction of the lethal dose.

That adding magnesium to barbital hastens recovery is probably due largely to the relative rapidity of both absorption and excretion of the smaller molecule. The "protective antagonism" may be accounted for both by the difference in time of peak effect, as well as by differences in the anatomical loci of toxicity. Of course, the direction of curvature of A indicates also some slight antagonism between the drugs as regards the narcotic threshold; certainly potentiation cannot be claimed.

CONCLUSIONS

1. Magnesium can be combined in a single injection with barbital in rabbits, in such a way as both to hasten the onset of narcosis and to lessen the persistence of this action, without evidence of significant increase in toxicity.

2. When two parts of magnesium chlorid are combined with one part sodium barbital, the minimal lethal dose is considerably higher than would be predicted on the basis of simple summation of effects.

REFERENCES

(1) Issekutz, B.: Therap. Monatshefte, 1915, xxix, 379.
(2) Loewe, S.: Klin. Wochenschr., 1927, vi, 1077.
(3) Käer, E., and Loewe, S.: Arch. exp. Pathol. u. Pharmakol., 1926, cxvi, 140.
(4) Käer, E., and Loewe, S.: Arch. exp. Pathol. u. Pharmakol., 1926, cxviii, 108.
(5) Barbour, H. G., and Winter, J. E.: Proc. Soc. Exper. Biol. and Med., 1928, xxv, 582.
(6) Winter, J. E., and Barbour, H. G.: Proc. Soc. Exper. Biol. and Med., 1928, xxv, 587.
(7) Winter, J. E., and Barbour, H. G.: Jour. Pharmacol. and Exper. Therap., 1931.
(8) Winter, J. E., Richey, C. H., and Barbour, H. G.: Jour. Pharmacol. and Exper. Therap., 1930, xxxviii, 343.
(9) Roemer, C.: Arch. exp. Pathol. u. Pharmakol., 1911, lxvi, 241.
(10) Trevan, J. W.: Proc. Roy. Soc., B, 1927, ci, 483.

VOPR. ONKOL.
14(7): 57-61

U51

Anticarcinogenic Action of Magnesium

K. A. Bazikyan and A. A. Akimov

Petrov Institute of Oncology and Yerevan Institute of Roentgenology, Radiology and Oncology

K. L. Bazikyan expressed the view that the low incidence of certain malignant tumors, especially stomach cancer, in Armenia is due to the abundance of magnesium salts in the soil, in water used for irrigation, and in rocks. By that time there were already published reports [1, 3, 6, 8, 9] suggesting a relationship between the presence of magnesium salts in the environment (soil, water, food products) and the frequency of certain malignant diseases, notably stomach cancer: the higher the magnesium content, the lower the level of these diseases. It was also noted that magnesium salts have a marked therapeutic effect on tumors [4, 5, 7, 10].

In view of the foregoing, we decided to study the antineoplastic effect of magnesium salts under experimental conditions. Experiments were performed on 259 mature male CC57BR mice. Skin papillomas were induced with 9,10-dimethyl-1,2-benzanthracene (DMBA). The mice were rubbed with a 0.1% benzene solution at the rate of 50 mg/kg per mouse every other day for a month. Connective-tissue tumors (sarcomas) were induced by a single subcutaneous injection of the animals with 3 mg of 1,2,5,6-dibenzanthracene (DBA). Magnesium chloride was used in all the experiments.

The effect of magnesium on the origin and development of papillomas was studied in 2 series of experiments (Table 1). In series 1, the animals were divided into 2 subgroups: control, which received ordinary

Table 1

Effect of Magnesium Chloride on the Origin of Skin Papillomas in Mice

1 Серия опытов	2 Подгруппы животных	3 Число мышей: в начале опыта 4	в конце опыта 5	пало в % 6	p
I	7 Контрольная	30	22	27	0,5
	8 Подопытная	30	24	20	
II	7 Контрольная	29	19	35	
	9 1 подопытная	30	24	20	0,3
	2 »	31	27	10	0,05
	3 »	29	17	42	

continuation
Продолжение

1 Серия опытов	2 Подгруппы животных	10 Число мышей с опухолями: 11 абсол.	12 в %	p	13 Среднее число опухолей на 1 мышь: 14 в абсолютных числах	15 % к контролю	p
I	Контрольная	18	82	0,5	1,1±0,16	100	
	Подопытная	16	66	0,3	0,80±0,11	73	0,1
II	Контрольная	14	74		0,90±0,17	100	
	1 подопытная	12	50	0,1	0,63±0,12	70	0,1
	2 »	11	41	0,05	0,55±0,14	51	0,07
	3 »	11	65	0,05	0,82±0,14	90	0,7

1 - Series of experiments

2 - Animal subgroups

3 - Number of mice

4 - at the start of the experiment

5 - at the end of the experiment

6 - died, %

7 - Control

8 - Experimental

9 - Experimental 1

10 - Number of mice with tumors

11 - abs.

12 - in %

13 - Average number of tumors per mouse

14 - abs.

15 - % of control

water throughout the experiment, and experimental, which drank water to which magnesium chloride had been added. The mice in this subgroup received magnesium 30 days from the first day the carcinogen was applied to the last.

In the second experiment, the animals were divided into 4 subgroups. The control was similar to that of the first experiment. Subgroup 1 received magnesium chloride prophylactically for 7 days and 30 days with the carcinogen. Subgroup 2 received magnesium chloride for 7 days prophylactically and 53 days from the time the benzene solution of DMBA was applied. Subgroup 3 received magnesium from day 8 after the first application of the carcinogen and for the next 48 days, i.e., until the first papillomas appeared.

The 0.1% benzene solution of DMBA was applied to the posterior portion of the spine of all the mice. The hair began to fall out after 3 or 4 applications, and by the time of the last application (total of 15) the treated spots were bald and in some cases even ulcerated. Judging by the control, the concentration of the carcinogen used was quite toxic.

Some of the mice died either during the time the carcinogen was applied or shortly after the last application.

In the second series of experiments in which we studied the effect of magnesium chloride on skin carcinogenesis, it exhibited definite antotoxic action, especially in experimental group 2 which received magnesium for a longer period of time (Table 1).

A completely different picture is presented by experimental group 3 in which 42% of the mice died. This was probably due to the fact that these animals did not begin to receive magnesium chloride until day 8 from the time the carcinogen was first applied.

Figures 1 and 2 show the dynamics of appearance of the papillomas. In all the groups of both experimental series, the first tumors appeared at almost the same time, between days 48 and 54, but they did not increase in each group at the same rate thereafter. In series 1, the experimental mice had fewer tumors than the control throughout the experiment. On day 200, the subgroup that received magnesium chloride included fewer mice with tumors and a smaller average number of tumors per mouse than the control.

In series 2, at first experimental subgroup 3 had the most tumors, but thereafter from day 75 the tumors ceased to appear and it was not until day 140 that they once again began to appear intensively. By day 200 subgroup 3 differed little from the control with respect to the number of tumors.

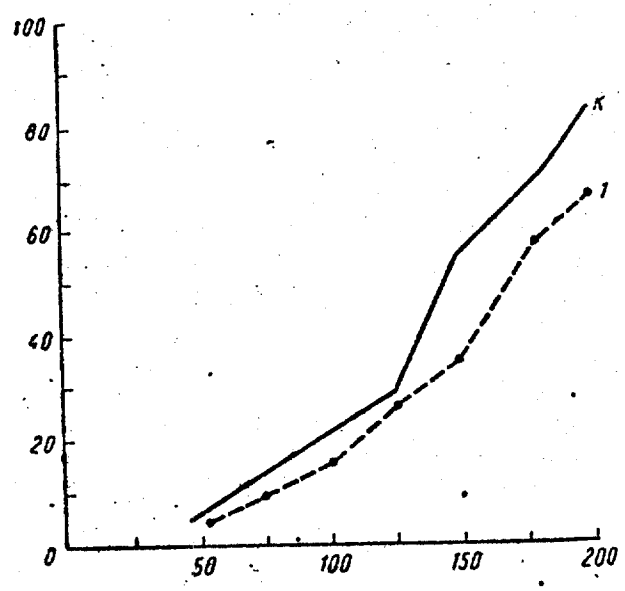


Fig. 1. Dynamics of development of skin papillomas in mice of experiment 1 K - control; 1 - mice that received a single dose of 60 mg/kg of magnesium chloride for 30 days.

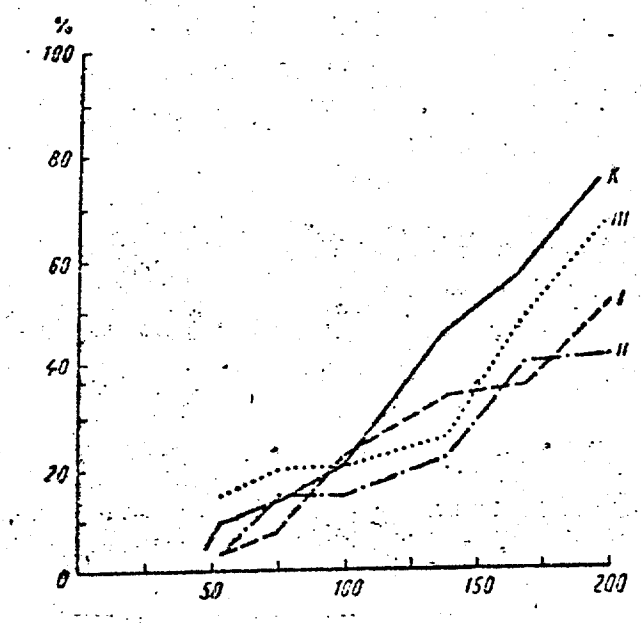


Fig. 2. Dynamics of development of skin papillomas in mice of experiment 2 K - control; 1 - first experimental group (magnesium chloride 7 days prophylactically and 30 days from the start of application of the carcinogen; II - second experimental group (magnesium chloride 7 days prophylactically and 53 days from the start of application of the carcinogen; III - third experimental group (magnesium chloride from day 8 after the first application of the carcinogen to the skin of the mice and for the next 48 days).

In experimental groups 1 and 2, the first tumors appeared the same day as in the control but from days 75 to 100 they appeared more slowly than in the control. By day 200 tumors developed in 74% of the control compared with 50 and 41% in experimental groups 1 and 2, respectively, and 65% in group 3.

Thus, of the groups of mice that received magnesium, the largest both in number of animals with skin papillomas and in number of tumors per mouse was subgroup 3. And the mice in this subgroup died more often. It is evident from the effects on experimental group 3 that to manifest antitoxic and antineoplastic action magnesium must be administered to mice prophylactically for several days before an experiment and at the same time that the carcinogen is first applied topically. It would appear, therefore, that the very first application of the carcinogen produces irreversible changes in the cells which later undergo malignant transformation.

The experimental group in series 1 and the first experimental group in series 2 occupy an intermediate position with respect both to the number of animals that died and to the mice with papillomas. There was no difference between the groups in either respect.

Thus, of the 2 experimental series with skin carcinogenesis, tumors appeared least frequently in the second experimental group of series 2, and it was in this subgroup, as noted above, that the fewest mice died. The reason may have been that they received magnesium longer than did the mice in the other groups.

In other experiments we studied the effect of magnesium chloride on the origin of tumors from connective tissue (Table 2). There were 3

Table 2

Effect of Magnesium Chloride on the Induction of Subcutaneous Sarcomas in Mice

Таблица 2

Влияние хлористого магния на индукцию подкожных сарком у мышей

1 Группы животных	2 Число мышей				6 Число мышей с опухолями		
	3 в начале опыта	4 в конце опыта	5 пало в опыте	P	7 абсол.	8 в %	P
9 Контрольная	30	18	40	0,3	15	83	
10 Первая подопытная	19	15	21	0,02	10	66	0,2
11 Вторая »	21	19	10	0,05	7	37	0,01

1 - Groups of animals

2 - Number of mice

3 - at the start of the experiment

4 - at the end of the experiment

5 - died, %

6 - Number of mice with tumors

7 - abs.

8 - in %

9 - Control

10 - Experimental 1

11 - Experimental 2

groups in this series. The control received tap water throughout the experiment. Experimental group 1 drank water to which magnesium chloride had been added for 30 days. Group 2 drank water with magnesium chloride throughout the experiment (200 days).

As in the preceding experiments, the first to die of the toxic action of 1,2,5,6-dibenzanthracene were the control mice, and by the end of the experiment this group had the greatest number of deaths. As in the experiments with skin carcinogenesis, the group in which the fewest animals died also included the smallest number of mice with tumors. Consequently, this experiment shows that magnesium can decrease the yield of subcutaneous sarcomas induced by 1,2,5,6-dibenzanthracene.

It is fair to conclude from our experiments that magnesium plays a definite part in the development of experimental tumors in animals.

Conclusions

1. Magnesium chloride added to the drinking water of mice at a daily dose of 60 mg/kg weakened the toxic action of both 9,10-dimethylbenzanthracene and 1,2,5,6-dibenzanthracene.

2. Under certain experimental conditions magnesium chloride markedly decreased the yield of skin papillomas induced by 9,10-dimethyl-1,2-benzanthracene and that of subcutaneous sarcomas induced by 1,2,5,6-dibenzanthracene.

Bibliography

1. Akimtsev, A.A., Pochvovedeniye (Soil Science), 1957, 7, 91.

2. Bazikyan, L.K., Tez. dokl. na IX Mezhdunar. protivorak. kongresse (Abstracts of papers read at the 9th International Cancer Congress), Tokyo, 1966.

3. Bukhman, P.I., Tr. Zakavk. konfer. khirurgov (Proceedings of the Transcaucasian Conference of Surgeons), Rostov-on-Don, 1937.

4. Kozhevnikova, L.I., in: Vopr. onkol. Konfer. nauch. stud. obshchestv (Aspects of Oncology. Conference of Science Students' Societies), Moscow, 1963.

5. Nagnibeda, N.I., Vopr. onkol. (Problems of Oncology), 1957, III(5):562.

6. Petrov, N.N., Zlokachestvennyye opykholi (Malignant Tumors), Leningrad-Moscow, 1948, 1.

7. Shilovtsev, S.P., et al., Tr. Kuybyshevskogo med. in-ta (Transactions of the Kuybyshev Medical Institute), 1964, 24:11.

8. Delbet, P., Presse medicale, 1928, 36, 93, 1473.

9. Tromp, S.W., Schweiz. all. Path. Bakt., 1955, 18(4):929.

10. Meyer, B.A., and J. Exner, Med. Press, 1953, 230(27):651.

69-320 ANTICARCINOGENIC EFFECT OF MAGNESIUM. (Rus.) Bazikian, K. A. (Petrov Res. Inst. Oncol., Leningrad, U.S.S.R.) and A. A. Akimov. Vop. Onkol. 14(7):57-61, 1968.

Magnesium chloride ($MgCl_2$; 60 mg/kg/day p.o.) reduced the toxic effect of 9,10-dimethyl-1,2-benzanthracene (DMBA; 50 mg/kg every 2 days for 1 mo., topically) and of 1,2,5,6-dibenzanthracene (DBA; 3 mg x 1 s.c.) as well as the yield of skin papilloma and s.c. sarcoma in mature male CC57BR mice. In mice treated with $MgCl_2$ + DMBA for 30 days, 24/30 survived for 200 days and 16 showed tumors (66%; av. 0.8/mouse), compared to 22/29 and 18/22 (82%; av. 1.1/mouse). In mice treated with $MgCl_2$ alone for 7 days (as prophylaxis) followed by 30 days with $MgCl_2$ + DMBA, 24/30 survived 200 days and 12 showed tumors (av. 0.63/mouse); in mice treated with $MgCl_2$ alone for 7 days, followed by DMBA for 53 days, 27/31 survived 200 days and 11 showed tumors (41%; av. 0.55/mouse). In mice treated for 48 days with $MgCl_2$, from day 8 after the initiation of treatment of DMBA (i.e., until the appearance of the first papillomas) 17/29 survived 200 days and 11 showed tumors (65%; av. 0.82/mouse), compared to 19/29 and 14/19 (74%; av. 0.9/mouse), resp., in controls. In mice admin. DBA and $MgCl_2$ (for 30 days), 15/19 survived 200 days and 10/15 (66%) developed s.c. sarcomas; in mice treated with $MgCl_2$ alone (200 days), 19/21 survived 200 days and 7 (37%) developed sarcomas compared to 18/30 and 15/18 (83%), resp., in controls.

02182

051

Vopr. Onkol., 14(7): 57-61 (1968)

ВОПРОСЫ ОНКОЛОГИИ, ТОМ XIV, № 7, 1968 г.

УДК 616-006-085.771.7 : 546.46

АНТИБЛАСТОМОГЕННОЕ ДЕЙСТВИЕ МАГНИЯ

К. А. Базикян и *А. А. Акимов*

Из лаборатории экспериментальной лекарственной профилактики и терапии злокачественных новообразований (зав.—засл. деят. науки РСФСР проф. Н. В. Лазарев) НИИ онкологии им. проф. Н. Н. Петрова (дир.—чл.-корр. АМН СССР проф. А. И. Раков) и из Ереванского института рентгенорадиологии и онкологии Армянской ССР (дир.—чл.-корр. АМН СССР В. А. Фанарджян)

К. Л. Базикян высказал мысль, что в Армянской ССР низкая заболеваемость злокачественными опухолями некоторых локализаций, и в частности раком желудка, обусловлена богатым содержанием солей магния в почвах, в используемых для орошения водах, в горных породах. В литературе [1, 3, 6, 8, 9] к этому времени также имелись сообщения о том, что между содержанием солей магния в окружающей среде (почва, вода, пищевые продукты) и частотой некоторых раковых забо-

леваний, в частности рака желудка, существует известная связь: чем выше содержание в среде солей магния, тем ниже уровень указанных заболеваний. Указывалось также и на благоприятный лечебный эффект солей магния при опухолевых заболеваниях [4, 5, 7, 10].

Исходя из приведенных выше данных мы задались целью изучить антибластомогенное действие солей магния в эксперименте.

Опыты проводились на 259 половозрелых мышах-самцах линии CC57BR. Кожные опухоли индуцировали 9,10-диметил-1,2-бензантраценом (ДМБА). Мышей смазывали 0,1% бензольным раствором из расчета 50 *мг/кг* на мышь через день в течение месяца. Опухоли из соединительной ткани (саркомы) мы получали путем одноразового введения под кожу мышей 3 *мг* 1, 2, 5, 6-дибензантрацена (ДБА). Во всех опытах использовался хлористый магний. Последний давался мышам в поилках из расчета 60 *мг/кг*, т. е. 15 *мг/кг* Mg^{++} ежедневно.

Влияния магния на возникновение и развитие кожных опухолей исследовалось в двух сериях опытов (табл. 1). В первой серии опытов животные были разбиты на две подгруппы: контрольную, получавшую на всем протяжении опыта обыкновенную воду, и подопытную, в которой к воде для питья добавляли хлористый магний. Мыши этой подгруппы получали магний 30 дней с первого дня смазывания животных канцерогеном до последнего.

Таблица 1

Влияние хлористого магния на возникновение кожных опухолей у мышей

Серии опытов	Подгруппы животных	Число мышей: в начале опыта	в конце опыта	пало в %	p
I	Контрольная	30	22	27	0,5
	Подопытная	30	24	20	
II	Контрольная	29	19	35	
	1 подопытная	30	24	20	0,3
	2 »	31	27	10	0,05
	3 »	29	17	42	

Продолжение

Серии опытов	Подгруппы животных	Число мышей с опухолями: абсол.	в %	p	Среднее число опухолей на 1 мышь: в абсолютных числах	% к контролю	p
I	Контрольная	18	82	0,5	1,1±0,16	100	
	Подопытная	16	66	0,3	0,80±0,11	73	0,1
II	Контрольная	14	74		0,90±0,17	100	
	1 подопытная	12	50	0,1	0,63±0,12	70	0,1
	2 »	11	41	0,05	0,55±0,14	51	0,07
	3 »	11	65	0,05	0,82±0,14	90	0,7

Во втором опыте животные были разбиты на четыре подгруппы. Контрольная была аналогична контролю первого опыта. Мыши первой подопытной группы получали хлористый магний 7 дней профилактически и 30 дней на фоне воздействия канцерогеном; второй— хлористый магний в течение 7 дней профилактически и на протяжении 53 дней от начала смазывания бензольным раствором ДМБА; наконец, в третьей подопытной группе мыши получали магний, начиная с 8 дня после первого нанесения на кожу животных канцерогена и на протяжении последующих 48 дней, т. е. до появления первых папиллом.

У всех мышей 0,1% бензольным раствором ДМБА смазывали заднюю часть спины; после 3—4 таких смазываний у мышей уже начинала выпадать шерсть, а к последнему нанесению канцерогена (15 смазываний) у всех животных на месте смазывания имелось облысение, а у части из них — даже язвы. Судя по контрольным животным, взятая нами концентрация канцерогена оказалась довольно токсичной для мышей.

Часть мышей погибла либо в период смазывания их канцерогеном, либо в первые же сутки после прекращения аппликации последнего.

Во второй серии опытов, в которой изучалось влияние хлористого магния на кожный канцерогенез, он проявил определенное антитоксическое действие, особенно во второй подопытной группе, где мыши получали магний в течение более длительного периода (см. табл. 1).

Совсем иную картину можно видеть в третьей подопытной группе, где погибло 42% мышей. Вероятно, это связано с тем, что животные этой группы стали получать хлористый магний только с 8 дня от начала дачи мышам канцерогена.

На рис. 1 и 2 видна динамика появления кожных опухолей. Во всех группах обеих серий опытов первые опухоли появились почти одновременно, а именно на 48—54 день, но в дальнейшем нарастание числа опухолей в каждой группе шло по разному. В первой серии в подопытной группе на протяжении всего эксперимента число мышей с опухолями было меньшим, чем в контроле. На 200 день опыта в подгруппе мышей, получавших хлористый магний, число мышей с опухолями, а также среднее число опухолей на мышь было меньшим, чем в контроле.

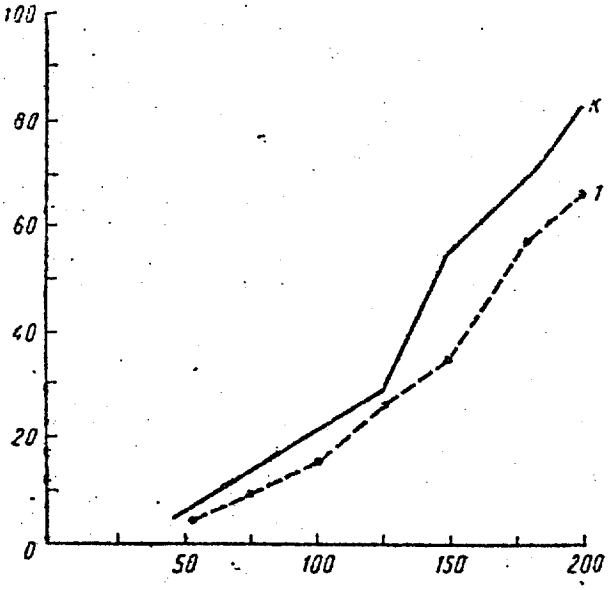


Рис. 1. Динамика возникновения кожных опухолей у мышей 1 опыта.

По вертикали — число мышей с опухолями в процентах; по горизонтали — время опыта в днях. К — контроль; 1 — мыши, получавшие хлористый магний в разовой дозе 60 *мг/кг* в течение 30 дней.

Во второй серии опытов вначале больше всего опухолей возникло у мышей третьей подопытной группы, но затем с 75 дня появление опухолей у мышей этой подгруппы приостановилось и лишь со 140 дня они стали интенсивно появляться вновь. К 200 дню опыта третья подгруппа по числу возникших опухолей не намного отличалась от контроля.

В первой и второй подопытных группах первые опухоли появились в те же дни, что и в контрольной группе, но уже с 75—100 дня их появление в этих группах шло менее интенсивно по сравнению с контролем. К 200 дню в контрольной группе опухоли возникли у 74% мышей, в первой и второй подопытных группах соответственно у 50—41% животных, а в третьей подопытной — у 65% мышей.

Итак, из групп, мыши которых получали магний, наибольшие как число животных с кожными опухолями, так и количество опухолей на одну мышь были в третьей подгруппе данного опыта. В этой же группе чаще отмечалась и гибель мышей. Из результатов третьей подопытной группы видно, что для проявления антитоксического и антибластомогенного действия магния важно вводить его мышам профилактически за несколько дней до опыта и одновременно с первыми смазывани-

ями животных канцерогеном, можно думать потому, что уже первая однократная дача канцерогена приводит к необратимым изменениям в клетках, которые в дальнейшем ведут к злокачественному росту.

Подопытная группа первой серии и первая подопытная группа второй серии занимают среднее положение по числу как погибших животных, так и мышей с кожными опухолями. При этом никакой разницы по этим показателям между данными группами нет.

Таким образом, из двух серий опытов с кожным канцерогенезом меньше всего опухолей возникло во второй подопытной группе второй серии; в этой же подгруппе, как ранее было отмечено, и погибло меньше всего мышей. Возможно, что такой результат зависит от более длительного, чем в других группах введения мышам магния.

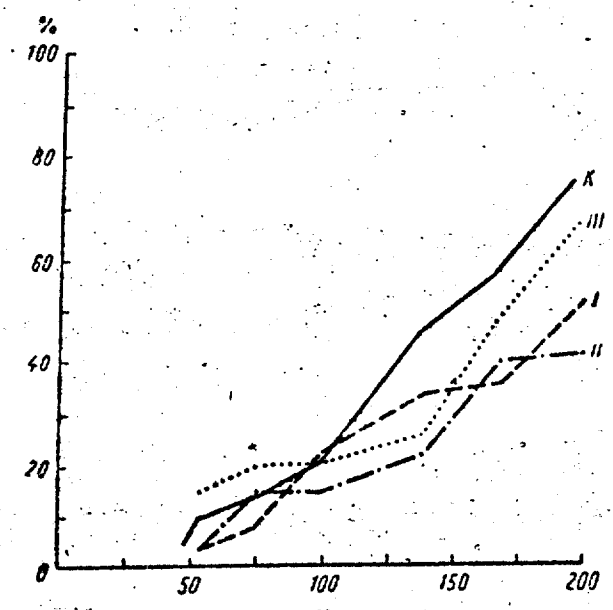


Рис. 2. Динамика возникновения кожных опухолей у мышей 2 опыта.

По вертикали — число мышей с опухолями в процентах; по горизонтали — время опыта в днях. К — контроль. I — первая опытная (хлористый магний 7 дней профилактически и 30 дней от начала смазывания канцерогеном); II — вторая опытная (хлористый магний 7 дней профилактически и 53 дня от начала смазывания канцерогеном); III — третья опытная (хлористый магний с 8 дня после первого нанесения на кожу мышей канцерогена на протяжении 48 дней).

В других опытах изучалось влияние хлористого магния на возникновение опухолей из соединительной ткани (табл. 2).

В этой серии опытов было три группы. Животные контрольной группы получали на всем протяжении опыта в поилках водопроводную воду, мышам первой подопытной группы добавляли к воде хлористый магний в течение 30 дней, а мышам второй подопытной группы — хлористый магний в течение всего периода опыта (200 дней).

Как и в предыдущих опытах, первыми от токсического действия 1, 2, 5, 6-дибензантрацена стали гибнуть мыши в контрольной группе. К концу опыта именно в этой группе погибло животных больше всего. Как и в опытах с кожным канцерогенезом, в группе, где погибло меньше всего животных, число

Таблица 2

Влияние хлористого магния на индукцию подкожных сарком у мышей

Группы животных	Число мышей: в начале опыта	в конце опыта	пало в %	P	Число мышей с опухолями: абсол.	в %	P
Контрольная	30	18	40	0,3	15	83	
Первая подопытная	19	15	21	0,02	10	66	0,2
Вторая »	21	19	10	0,05	7	37	0,01

мышей с опухолями было наименьшим. Следовательно в данном опыте можно было увидеть, что магний способен уменьшить выход подкожных сарком, индуцируемых 1, 2, 5, 6-дибензантраценом.

На основании наших экспериментов можно сказать, что магний играет определенную роль в возникновении экспериментальных опухолей животных.

Выводы

1. Хлористый магний, вводившийся мышам в поилках с питьевой водой из расчета суточной дозы 60 *мг/кг*, ослаблял токсический эффект как 9, 10-диметилбензантрацена, так и 1, 2, 5, 6-дибензантрацена.

2. При определенных условиях опыта хлористый магний значительно уменьшал выход кожных опухолей, индуцируемых 9, 10-диметил-1, 2-бензантраценом, или подкожных сарком, вызываемых 1, 2, 5, 6-дибензантраценом.

ЛИТЕРАТУРА

1. Акимцев А. А. Почвоведение. 1957, 7, 91.
2. Базик'ян К. Л. Тез. докл. на IX Междунар. противорак. конгрессе. Токио, 1966.
3. Бухман П. И. Тр. Закавк. конфер. хирургов. Ростов-на-Дону, 1937.
4. Кожевникова Л. И. В сб.: Вопр. онкол. Конфер. науч. студ. обществ. М., 1963.
5. Нагнибеда Н. И. Вопр. онкол. 1957, III, 5, 562.
6. Петров Н. Н. Злокачественные опухоли. Л.—М., 1948, 1.
7. Шиловцев С. П. и др. Тр. Куйбышевского мед. ин-та, 1963, 24, 11.
8. Delbet P. Presse médicale, 1928, 36, 93, 1473.
9. Tromp S. W. Schweiz. all. Path. Bakt., 1955, 18, 4, 929.
0. Meyer B. A., Exner J. Med. Press, 1953, 230, 27, 651.

ANTIBLASTOMOGENIC ACTION OF MAGNESIUM

K. A. Bazikyan and *A. A. Akimov*

The N. N. Petrov Research Institute of Oncology and Institute of Roentgenoradiology and Oncology of the Armenian SSR

The experiments were carried out on 259 adult male mice of the line CC57Br. Skin cancers were induced by 9,10 — dimethyl — 1,2 — benzanthracene (DMBA), and subcutaneous sarcomas — by 1, 2, 5, 6,— dibenzanthracene (DBA). The injection of magnesium chloride in mice, the daily dose of 60 mg/Kg during 30—50 days since the initial application of carcinogen, reduced the incidence of skin tumors by 30 per cent, whereas in cases when magnesium chloride was injected in animals also for prophylactic purposes within a 7 day period then the incidence of cancers decreased by 49 per cent.

Also, magnesium chloride inhibited the development of subcutaneous sarcomas. In a series where the animals were injected magnesium during 200 days tumors occurred only in 37 per cent of cases, while in control series — in 83 per cent

Magnesium Intoxication in a Premature Infant

Magnesium sulphate was first used for the treatment of eclampsia in 1906[1] and was rediscovered in 1925.[2] Recent reviews have emphasized that it is the drug of choice in the treatment of acute pre-eclampsia[3,4] and has no toxic effects on the fetus.[3] The following is the first report of an infant in whom prolonged magnesium toxicity was reversed by an exchange transfusion.

Case History

On April 24, 1966, an acutely pre-eclamptic 21-year-old primigravida (46 kg) was admitted in active labor at 30 weeks' gestation. She was hyperreflexic and her blood pressure rose rapidly from 140/100 to 160/110. She was sedated with morphine sulphate, 8 mg subcutaneously, and magnesium sulphate. She received 3 gm magnesium sulphate intravenously 3½ hours, 6 gm intramuscularly 2½ hours, and 2 gm intravenously ½ hour prior to precipitous vertex delivery of a flaccid female infant (1.6 kg). The baby was apneic and cyanotic and had a heart rate of 120 (Apgar score 2 at 1 minute of age). Endotracheal intubation was performed at once; she was then ventilated, given 0.1 mg levallorphan tartrate (Lorfan) intravenously and 3 ml of 0.88 mEq/ml sodium bicarbonate intravenously. Penicillin, 60,000 units intramuscularly, and kanamycin, 20 mg intramuscularly, were administered daily for 8 days. Ten percent dextrose and water, 50 cc/kg/24 hours, plus 4.1 ml of 0.88 mEq/ml sodium bicarbonate were infused via the umbilical vein. One hour after birth, when there were still no spontaneous respirations, movements, reflexes or withdrawal to painful stimuli, she was placed on the Bird Respirator (Mark VII with infant circle). By 6 hours of age she could be maintained off the respirator, although her breathing was slow and shallow and she was still paralyzed (see Table I and Figure 1). At 9 hours of age a catheter was placed in one umbilical artery to permit blood gas sampling. Presumptive diagnosis at this time was cerebral birth injury, although congenital myasthenia gravis, amyotonia congenita, or magnesium toxicity were also considered. A tensilon test (edrophonium chloride, 0.1 mg subcutaneously) produced only brief spontaneous movements of the fingers and toes, indicating that myasthenia was not her primary disease. Calcium gluconate (50

Pediatrics
40:100-103
(1967)

mg intravenously) also produced a similar response. At 14½ hours of age she became apneic again and deeply cyanotic, and she required further assisted ventilation (Table I).

Her serum magnesium, measured by a fluorometric method,[5] was 15 mEq/l at 9 and again at 16 hours of age, but it had fallen to 9.8 mEq/l by 24 hours of age. At this time she received an exchange transfusion using 500 ml of B positive ACD blood (magnesium level 1.4 mEq/l). After 280 ml of blood had been exchanged, she started to move and kick; at the same time she cried and breathed spontaneously (Table I) and had to be restrained in order to complete the exchange transfusion. Her subsequent course was uncomplicated apart from a mild chest infection.

No signs of magnesium toxicity were observed in the mother, who was hyperreflexic during labor. On the third postpartum day her creatinine and serum magnesium were normal. Unfortunately, no magnesium level was obtained before this time.

Since her initial illness the baby has progressed well (Fig. 2) and was developing normally when last seen at 6 months of age.

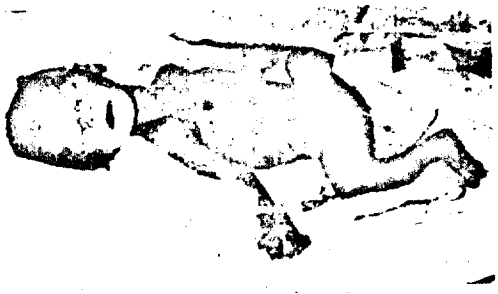


Fig. 1. Patient when 11 hours old. Note the frog position of her extremities and her open mouth from lack of muscle tone.

Fig. 2. Patient at age 3 months.

Comment

Magnesium has a dual effect on the nervous system. In high concentrations it causes peripheral neuromuscular block and central depression.[6,7] Magnesium exerts a curare-like action at the motor end-plate causing decreased liberation of acetylcholine and muscular paralysis. This effect is antagonized by excess calcium or physostigmine. The anesthetic properties of magnesium were discovered in 1906,[7] but the lethal and anesthetic levels were so close its use was abandoned. High levels of magnesium also depress smooth muscle contraction. The effect on vascular smooth muscle results in hypotension.[6] In normal man and animals, drowsiness, hypotension, and bradycardia usually occur when the serum magnesium level exceeds 7 mEq/l; absent tendon reflexes and prolonged atrioventricular conduction at 10 mEq/l; respiratory failure at 15 mEq/l; and cardiac arrest at 24 mEq/l. In chronic renal disease toxic symptoms occur at lower serum levels.[7]

Because parenteral magnesium is rapidly excreted magnesium toxicity is rarely seen. In patients with pre-eclampsia Chesley and Tepper found a serum level of 4.4 mEq/l immediately following an intravenous injection of magnesium sulphate, and 5.0 mEq/l 2 hours after an intramuscular injection of 10 gm. Thus, in our patient, peak serum levels from the mother's first intramuscular dose could have coincided with the peak serum level from her second intravenous dose. It is interesting that Aikawa[8] observed that tagged magnesium was actively concentrated in the placenta of the pregnant rabbit, and was rapidly taken up by fetal tissues. If this mechanism also occurs in the human it might account for the high fetal level in our baby. Although Chesley and Tepper[5] found that fetal magnesium levels (cord) never exceeded 96% of the maternal level in our

TABLE I

HOSPITAL COURSE

Age in hours	*0*	*6*	*9*	*12*	*14*	*14½*	*16*	*20*	*24*	*24½*	*25*	*25½*	*30*	*35*	*56*	*80*	*86*	*120*	*15 days*
Spontaneous movements	0	0	0	0	0	0	0	0	0	+	+	+	+	+	−	−	−	−	+
Assisted ventilation	+	0	0	0	0	+	+	+	+	0	0	0	0	0	0	0	0	0	0
Respiratory rate (spontaneous) breaths/minute	0	20	20	20	14	0	0	0	0	1st Cry	Crying	44	36	60	44	44	40	40	
Heart rate	120	120	108	110	108	114	116	118	112	116	114	126	124	128	136	136	140	140	
Direct arterial blood pressure mm Hg								42/24	38/20							52/36			
Serum magnesium mEq/l			15				15		9.8	7.5	5.9	5.4			5.3	5.3		3.2	1.3
PO_2* mm Hg			57	100	168		330	393	340	440		65	330	318	113				
Inspired oxygen concentration %	100	40	21	High	High	100	100	100	100	100		21	70	90	38	24	21	21	21
PCO_2* mm Hg			57	58	60		19	18	38	34		46	43	52	45	41			
pH*			7.25	7.23	7.23		7.61	7.63	7.33	7.34		7.25	7.45	7.40	7.39	7.39			
									Exchange Transfusion 500 cc B+ACD blood										

* Corrected to body temperature.

infant the amniotic fluid level was 4.7 mEq/l while the maximum maternal level was 4.2 mEq/l, suggesting active concentration by the fetus.

Fishman[10] reported 20-minute respiratory depression at birth in an infant whose mother had received excessive intravenous magnesium sulphate; the infant had a serum level of 3.4 mEq/l at 4 hours of age when he was asymptomatic but presumably he had higher levels at birth. In our patient, muscular paralysis, absent reflexes, and respiratory failure occurred with hypermagnesemia, with a dramatic recovery during an exchange transfusion. It is interesting that Stern and co-workers[11] have shown that ACD blood decreases free magnesium by increasing citrate-binding, so that the exchange transfusion may have had a beneficial effect by increasing magnesium binding as well as decreasing the total amount in the circulation.

Summary

This report of a premature infant with hypermagnesemia indicates that magnesium sulphate therapy for pre-eclampsia may have profound effects on the fetus. In this infant the toxic effects of motor and respiratory paralysis were immediately reversed when the serum magnesium level was lowered during an exchange transfusion.

June P. Brady, M.B., B.Chir.
H. C. Williams, B.S.
San Francisco General Hospital
Department of Pediatrics
University of California
School of Medicine
San Francisco, California 94110

This work was supported in part by the San Francisco Heart Association, by the Heart Association of the Redwood Empire, and by the University of California Committee on Research, and Fund.

Dr. Brady is an Advanced Research Fellow of the American Heart Association supported in part by the Alaska Heart Association.

References

1. Hagen, E.: To tilfaelde af eclampsia gravidarum behandlet med sulfas magnesicus injiceret i ryggmarvens subarachnoidalrum. Medicinsk Revue Bergen, 23:264, 1906, cited in Chesley, L. C., and Tepper, I.: Plasma levels of magnesium attained in magnesium sulphate therapy for pre-eclampsia and eclampsia. Surg. Clin. N. Amer., 37:353, 1957.
2. Lazard, E. M.: A preliminary report on the intravenous use of magnesium sulphate in puerperal eclampsia. Amer. J. Obstet. Gynec., 9:178, 1925.
3. Chesley, L. C., and Tepper, I.: Plasma levels of magnesium attained in magnesium sulphate therapy for pre-eclampsia and eclampsia. Surg. Clin. N. Amer., 37:353, 1957.
4. Flowers, C. E., Jr.: Magnesium sulphate in obstetrics. A study of magnesium in plasma, urine, and muscle. Amer. J. Obstet. Gynec., 91:763, 1965.
5. Schachter, D.: The fluorometric estimation of magnesium in serum and in urine. J. Lab. Clin. Med., 54:763, 1959.
6. Engback, L.: The pharmacological actions of magnesium ions with particular reference to the neuromuscular and the cardiovascular system. Pharmacol. Rev., 4:396, 1952.
7. Randall, R. E., Jr., Cohen, D., Spray, C. C., Jr., and Rossmeisl, E. C.: Hypermagnesemia in renal failure. Ann. Intern. Med., 61:73, 1964.
8. Meltzer, S. J., and Auer, J.: Physiological and pharmacological studies of magnesium salts. II. The toxicity of intravenous injections; in particular the effects upon the centres of medulla oblongata. Amer. J. Physiol., 15:387, 1906.
9. Aikawa, J. K., and Brums, P. D.: Placental transfer and fetal tissue uptake of Mg^{28} in the rabbit. Proc. Soc. Exper. Biol. Med., 105:95, 1960.
10. Fishman, R. A.: Neurological aspects of magnesium metabolism. Arch. Neurol., 12:562, 1965.
11. Stern, L., Denton, R. L., Bajpai, P. C., and Sugden, D.: Serum ionic magnesium in exchange transfusions. J. Pediat., 69:966, 1966.

JOURNAL OF THE MALAYA BRANCH,
BRITISH MEDICAL ASSOCIATION.
Vol. 3. No. 1. June, 1939.

FATAL RESULTS FOLLOWING THE ADMINISTRATION OF MAGNESIUM SULPHATE.

BY

F. E. BYRON, Ph.D., B.Sc., F.I.C.,

Acting Chief Chemist, Institute for Medical Research, Kuala Lumpur.

During the past three years, the viscera of five young Tamil children have been examined by the Chemical Division of the Institute for Medical Research with a view to determining the possibility of death from poisoning.

A brief history of each of the five cases is given below:

(a) Male Tamil child, age 10 years, treated by estate dresser with mist. alb. (dose not stated). No anthelmintics given. The child collapsed and died within two hours of receiving treatment.

(b) Male Tamil child, age 9 years, treated by estate dresser with 5 minims of chenopodium oil and ½ ounce of mist. alb. fort. The child collapsed and died within 75 minutes of receiving treatment.

(c) Male Tamil child, age 2½ years, treated by estate dresser with 6 fluid drachms of mist. alb. No anthelmintics given. The child collapsed and died within 45 minutes of receiving treatment.

(d) Male Tamil child, age 10 years, treated by estate dresser with 1¼ ounces of mist. alba. No anthelmintics given. The child collapsed and died within 1½ hours of receiving treatment.

(e) Male Tamil child, age 2½ years, treated by estate dresser with 2 minims of chenopodium oil and 6 fluid drachms of a 50% solution of magnesium sulphate in water. The child collapsed and died ten minutes after receiving treatment.

The histories of all the above cases are very similar, being characterised by the rapidity with which death followed the administration of magnesium sulphate, and the fact that no post-mortem abnormalities, apart from general congestion, were noted.

Toxicological examination failed to reveal the presence of poison in any of the viscera. The first two cases were spread over a period of two years and no suspicions as to the possibility of magnesium sulphate poisoning were aroused. The last three cases were all received within a period of two months and, in the absence of any other cause of death, attention was drawn to the possibility of poisoning by the magnesium sulphate, in spite of the fact that this salt, either in simple solution or as *mistura alba*, is one of the commonest of purges. Death from chenopodium oil can be eliminated, as only two of the five children received any.

It is well known that magnesium salts, if injected intravenously, may cause coma and death, but when administered by mouth, absorption takes place very slowly from the intestine and elimination is fairly rapid from the urine so that a dangerous concentration of magnesium does not occur and no toxic systemic action takes place. It seems possible, however, that in certain cases, following oral administration, especially in strong solutions, that absorption can take place to such an extent that toxic symptoms result.

Thatcher, Harvey S. (1928) gives details of a death from a massive dose of magnesium sulphate. In this case two ounces of magnesium sulphate were recovered from the stomach contents. Thatcher also refers to a number of other cases of poisoning from magnesium sulphate, but unfortunately the references are not available in the Institute library.

Hirschfelder (1934) shows that the magnesium content of the plasma of nephrectomised dogs rose rapidly after the oral ingestion of magnesium sulphate and, if the dose was large enough, coma and death ensued. He also found that the plasma magnesium content of patients suffering from renal diseases rose dangerously after administering clinical doses of magnesium sulphate.

In the last case of the Tamil child, aged 2½ years, a sample of the blood was sent and was found on analysis to contain 8 milligrams of magnesium per 100 cubic centimetres of blood serum. Normal blood serum contains 2 to 3 milligrams per 100 cubic centimetres of serum. It is obvious, therefore, that abnormal absorption had occurred. Hirschfelder found concentrations of 9 to 11 milligrams of magnesium per 100 cubic centimetres in adult nephritic patients without signs of coma, but it seems likely that in the case of a young child this concentration might be fatal.

The recommended dose of mist. alb. is ½ to 3 fluid ounces, of mist. alb. fort ½ to 1 fl. ounce, and of magnesium sulphate 30 to 90 grains. These are doses for adults, so that the doses given in the five cases quoted did not err on the side of safety.

REFERENCES

Thatcher, Harvey S., *Jl. Am. Med. Assoc.* 1928, vol. 91, 1185.
Hirschfelder (1934) *Jl. Am. Med. Assoc.* 1934, vol. 102, 116.

AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 214, No. 2, February 1968. *Printed in U.S.A.*

Effects of magnesium ions on calcium and phosphorus metabolism[1,2]

IRWIN CLARK[3]
Departments of Orthopedic Surgery and Biochemistry,
College of Physicians and Surgeons, Columbia University, New York Ci

CLARK, IRWIN. *Effects of magnesium ions on calcium and phosphorus metabolism.* Am. J. Physiol. 214(2): 348–356. 1968.—The effects of supplemental but nontoxic amounts of orally administered magnesium ions on calcium and phosphate metabolism of adult male rats fed diets containing varied amounts of calcium and phosphorus were studied. A 12-day balance period was investigated after the animals were habituated to their respective diets for 5 days. If the diets contained adequate amounts of calcium, supplemental magnesium decreased fecal calcium (increased absorption) and increased urinary calcium irrespective of the dietary level of calcium. The net effect of supplemental magnesium was to increase calcium balance. The mechanism for the increased absorption of calcium from the intestinal tract or the hypercalciuria is not known. Supplemental magnesium had no effect on phosphorus absorption when dietary phosphorus was suboptimal; it increased it when the dietary calcium-to-phosphorus ratio was low. The effects of magnesium on phosphate absorption appear to be the result of a magnesium-calcium interaction. Urinary phosphorus decreased with increasing magnesium intake. In general, magnesium increased phosphorus balance primarily as a result of renal conservation of phosphorus.

balance; excretion; dietary calcium-to-phosphorus ratio; serum values

---

IT HAS BEEN KNOWN since the turn of the century that the parenteral administration of magnesium ions produces hypercalciuria in dogs, cats, and rabbits (27), a finding which has been confirmed in man (41) and rat (9). Injection of magnesium into the renal artery (7) or intravenously (33) causes an almost immediate hypercalciuria which suggests a direct effect of magnesium ions on the kidney. It has been suggested (33) that there exists a common reabsorptive site for calcium and magnesium in the kidney tubules and that large amounts of magnesium ions inhibit the reabsorption of calcium. Also in vitro studies with the everted gut sac have shown tha magnesium ions inhibit the active transport of calcium ions (18, 35) and it has been postulated there also exis in the intestine a common absorptive site for calcium and magnesium, and that these ions compete for th site. Further evidence for competition between these ion comes from studies of magnesium deficiency, where i has been shown that calcium absorption from the intestine is significantly enhanced (1) suggesting that th presence of magnesium ions inhibits calcium absorption. Experimental (24, 42, 43) and naturally occurrin (37) magnesium deficiencies have been extensively in vestigated. However, interpretations of the effects magnesium ions on calcium metabolism based on such studies may not be valid since the deficient animal is sick with pathologic changes in tissues (24) which ma alter cell permeability. Thus, the increase seen in calcium absorption (1) may therefore be the result these tissue changes rather than a direct effect of magnesium ions.

In contradistinction to these results, studies in the have shown that magnesium ions per os markedly en hance the intestinal absorption of calcium and strontium at the same time they induce hypercalciuria (8, 1 suggesting that magnesium ions affect the kidney an intestines differently.

Disagreements also exist with respect to the effects magnesium on over-all calcium metabolism. Publishe reports concerning the effects of magnesium ions calcium balance vary from those which claim that hig magnesium intake decreases (16) or has little effect calcium balance (4, 14) to those which claim it increas calcium balance (6, 10, 11, 17). However, none of the studies are strictly comparable, since different dietar conditions and species were employed. Further, in studi designed to determine the mechanism whereby mag nesium ions promote the elimination of skeletally bou strontium, it was observed that the effect of magnesiu on calcium balance was dependent to a large extent the dietary ratios of calcium and phosphorus (11).

Magnesium ions also influence the absorption excretion of phosphorus. It has been demonstrated t

Received for publication 15 April 1967.
[1] The studies were supported by US Atomic Energy Commission Contract AT(30-1)2530 and Public Health Service Grant AM 04071.
[2] A preliminary report of this work has been presented, *Federation Proc.* 24: 372, 1965.
[3] Recipient of Career Development Award 5-K3-AM-15, 374-04.

magnesium ions depress urinary phosphorus (7, 10) and may increase or decrease phosphorus absorption, depending on the dietary calcium-to-phosphorus ratios (8, 11). The growth of rats fed a high magnesium intake is also dependent on the dietary levels of phosphorus (15). Further, high dietary levels of phosphorus accentuate the symptoms of magnesium deficiency (32) and also increase the minimal requirements for magnesium (30, 31). Thus, there appears to be significant interrelations between dietary phosphorus and magnesium as well as between calcium and magnesium.

The following experiments were designed to evaluate the effects of supplemental but nontoxic amounts of magnesium on calcium and phosphorus excretion. By varying the dietary levels of calcium and phosphorus, magnesium-calcium and magnesium-phosphorus interactions on calcium and phosphorus metabolism also were studied.

MATERIALS AND METHODS

Adult male Holtzman rats weighing between 275 and 295 g were divided into 12 groups of 12 animals each. The animals received the following basic diet which contained: 60% cerelose, 24% blood fibrin, 2% Alphacel, 10% Mazola oil, 4% salt mix, and adequate amounts of all vitamins. Blood fibrin was used rather than casein, since the latter protein contains relatively large and variable amounts of phosphorus. All diets contained the same amounts of magnesium (0.05%), and trace elements which are present as follows (per kg of diet): ferric citrate (1,100 mg), $ZnSO_4 \cdot 7H_2O$ (240 mg), $CuSO_4 \cdot 5H_2O$ (160 mg), $MnSO_4 \cdot H_2O$ (980 mg), KI (1.2 mg), and NaF (40 mg). Each group of animals was fed a diet containing different percentages of calcium and phosphorus (Table 1). Variations in calcium-to-phosphorus ratios were achieved by using various salts of these elements. Adjustments in the total quantity of salt mix to keep it as close as possible to 4% of the diet were made by altering the amount of Alphacel whenever necessary, e.g., adding Alphacel to salt mix to make it equal 4% of diet. When the salt mix was more than 4% of diet, Alphacel in the amount of excess salt was subtracted to maintain the same percents of fat, carbohydrate, and protein.

TABLE 1. *Dietary calcium-to-phosphorus ratios*

Percent Dietary Phosphorus		Percent Dietary Calcium			
		0.2	0.4	0.8	1.6
0.8	KH_2PO_4	14.5	14.5	8.0	8.0
	Na_2HPO_4			3.7	3.7
	$NaH_2PO_4 \cdot H_2O$	7.1	7.1		
	$CaH(PO_4)_2 \cdot H_2O$	12.6	12.6	23.5	
	$Ca_3(PO_4)_2$				28.9
	$CaCO_3$		5.0	10.7	12.0
0.4	K_2HPO_4			8.0	8.0
	KH_2PO_4	10.5	10.5		
	$KHCO_3$	1.5	1.5		
	Na_2HPO_4			3.7	3.7
	$NaH_2PO_4 \cdot H_2O$	7.2	7.2		
	$CaH(PO_4)_2 \cdot H_2O$			7.3	7.2
	$CaCO_3$	5.0	10.0	17.1	37.3
0.2	K_2HPO_4	5.2	5.2	6.7	6.7
	$KHCO_3$	5.4	5.4		
	KCl			1.1	
	$NaH_2PO_4 \cdot H_2O$	3.6	3.6		
	$NaHCO_3$	2.2	2.2		
	Na_2HPO_4			3.7	3.7
	$CaCO_3$	5.0	10.0	20.0	40.0

After the 12 groups of rats were habituated to their respective diets for 5 days, each group of 12 rats was divided into 2 subgroups of 6 animals. One group had $MgCl_2 \cdot 6H_2O$ added to its drinking water to give a final concentration of 2% $MgCl_2 \cdot 6H_2O$ (w/v). To make the magnesium solution more palatable and to ensure an adequate volume of urine, glucose to a final concentration of 10% (w/v) was added to the drinking water of all rats. Since the control rats tended to drink more of the sweet glucose solution than did the magnesium-supplemented rats, all animals received 40 ml of drinking solution and the magnesium-supplemented rats usually drank between 30–40 ml daily while the controls drank it all. Following the adjustment period to the different diets, a 12-day balance study was begun, during which time, food and fluid consumptions were measured daily and urine and feces collected every 2 days. The control animals consumed approximately 50 mg of magnesium during the balance period, i.e., 100 g food, while the magnesium-supplemented rats consumed from 800 mg to 1,000 mg. Occasionally the rats supplemented with magnesium developed loose stools and diarrhea for the first few days. Food intake was controlled so that the control rats and those supplemented with magnesium ate approximately the same amount during the balance period. Spillage of food was taken into account in the calculation of food consumption. During the balance period, each rat was kept in an individual stainless steel metabolism cage designed to separate urine from feces. To facilitate the collection of urine, the funnel portion of the cage was coated with paraffin. After the fecal matter was removed, the cages were rinsed with distilled water to remove any urine that might have remained on the cages. The washings were pooled with the urine and made up to a known volume.

Calcium determinations were made by "reverse" flame photometry (25) on urine directly and on feces following ashing at 650 C. Phosphorus was determined by a modification of the Fiske-SubbaRow procedure (21). Univariate (36) and multivariate (2) analyses of variance were made on all data. Statistical results from the multivariate analyses of variance are not reported since they provided no additional information. The data from 13 of the rats are not included in the analyses since these animals developed severe respiratory ailments, or their urine or fecal samples were lost.

RESULTS

Dietary and fecal calcium and phosphorus. It has been observed in previous studies that magnesium-supplemented rats tended to eat more food than their controls (11). Consequently, during the balance period, the magnesium-supplemented animals were pair-fed to their respective controls with the exception of the "pair-fed" magnesium-supplemented group, i.e., 0.8% phosphorus and 1.6% calcium which inadvertently was allowed to eat more food than its control (Table 1).

Provided the diets contained at least 0.4% calcium, supplemental magnesium decreased fecal calcium significantly (Fig. 1). The analysis of variance shows a significant magnesium main effect on fecal calcium as well as significant two-way interactions between calcium-magnesium and phosphorus-magnesium (Table 2). Since the three-way interaction is much less significant than either two-way interaction, the latter interactions must work in opposite directions.

Supplemental magnesium had no effect on fecal phosphorus when dietary level of phosphorus was low (0.2%) regardless of the level of dietary calcium. It decreased when diet contained 0.4% phosphorus and increased when dietary phosphorus was high (0.8%) and calcium was low (0.2%) (Fig. 2).

Urinary calcium and phosphorus. Supplemental magnesium increased significantly urinary calcium by all groups (Fig. 3). Analysis of variance (Table 2) shows significant magnesium-calcium and magnesium-phosphorus interactions. Since the three-way interaction

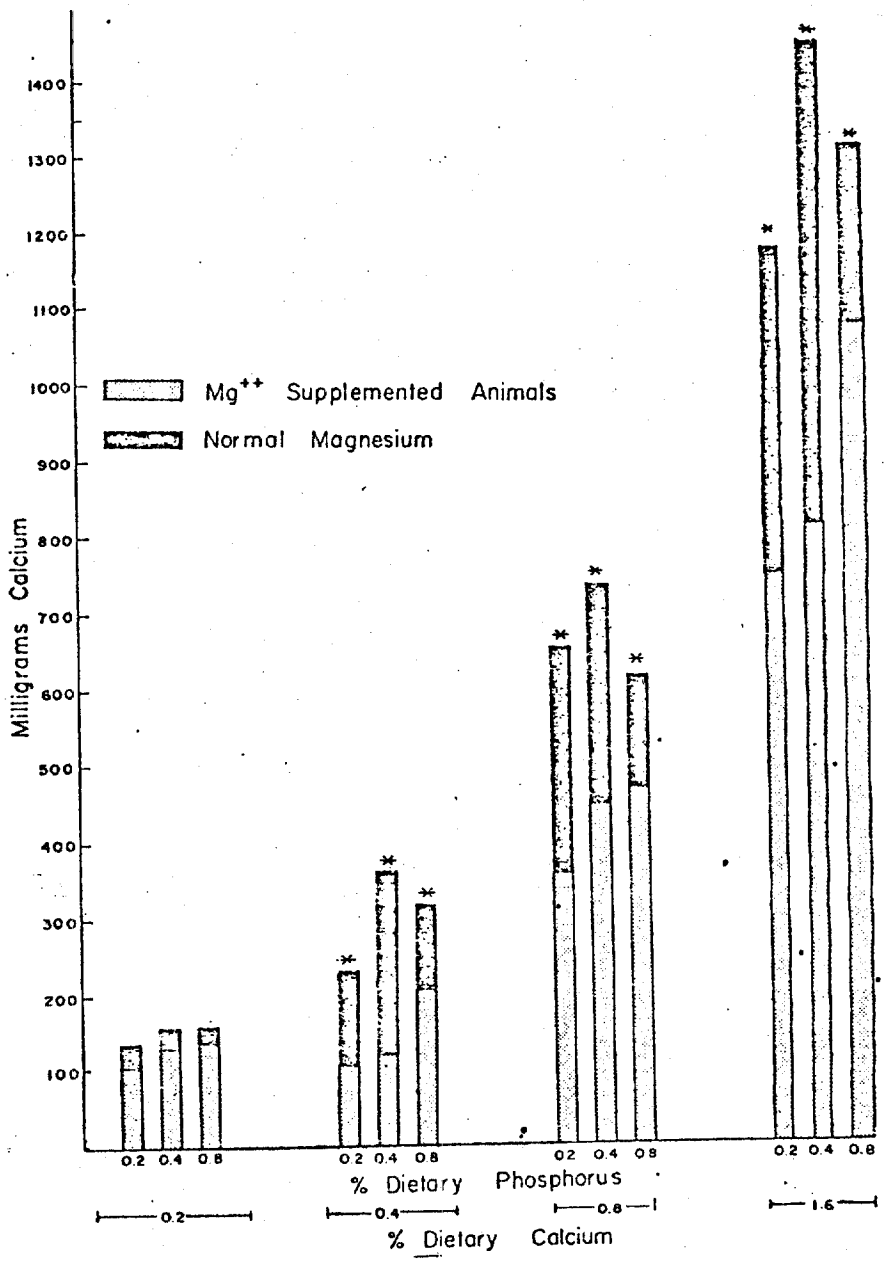


FIG. 1. Total fecal calcium—12 days. Asterisks (*) indicate a significant difference ($P < .01$) between rats fed normal diets and those fed supplemental magnesium. The height of the bar represents excretion by group of rats, e.g., control or magnesium supplemented depending on the shading of the top portion. The line dividing the two shadings represents the total excretion by the other group. For example, in the bar representing rats fed 1.6% calcium and 0.8% phosphorus the top of the bar shows that rats fed normal magnesium excreted about 1,300 mg while rats fed supplemental magnesium excreted less than 1,100 mg (the junction between the two shadings).

TABLE 2. *P values for the univariate analysis of variance*

	Fecal		Urinary		Serum		Balance	
	Ca	P	Ca	P	Ca	P	Ca	P
Mg main effect*	<.001	NS	<.01	<.001	<.05	NS	<.001	<.001
Mg, P interaction†	<.001	<.05	<.001	<.001	<.01	<.001	<.001	<.001
Mg, Ca interaction	<.001	<.001	<.001	<.001	NS	<.06	<.001	<.06
Mg, P, Ca interaction	<.02	<.02	<.02	<.001	NS	.002	NS	.005

NS = not significant. * Main effect measures the changes in the responses caused by altering this factor irrespective of changes in any other factors, e.g., calcium or phosphorus. † Interaction is a measure of the degree of mutual dependence between factors and if the *P* values are low (<.01) this would indicate a high degree of interdependence.

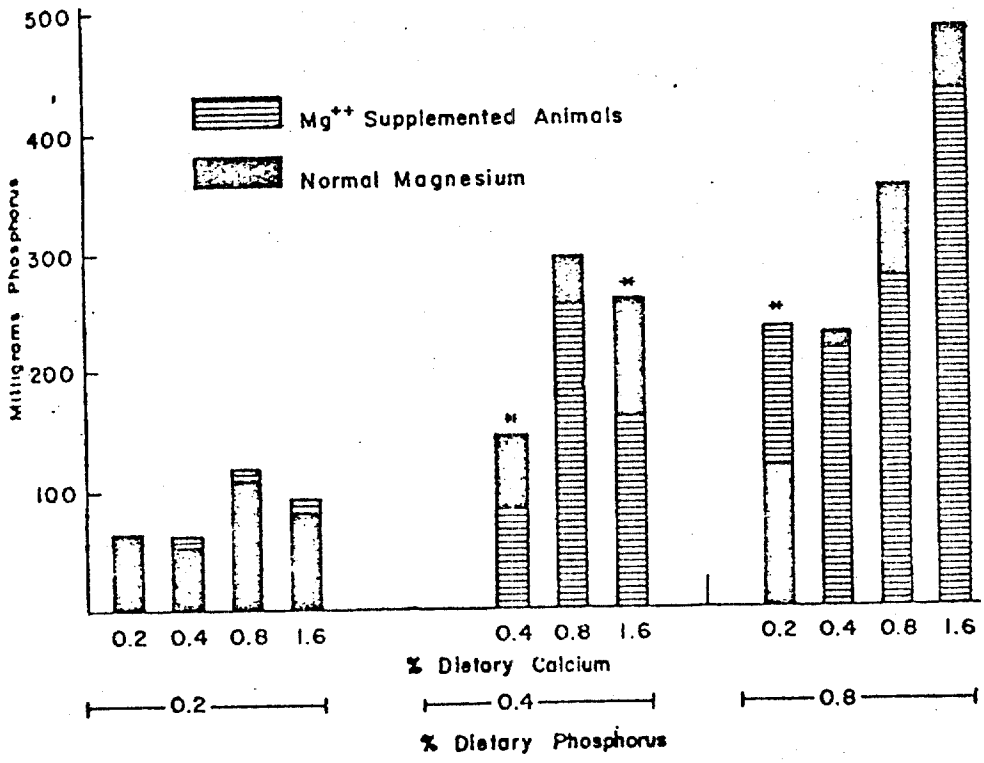


FIG. 2. Total fecal phosphorus—12 days. Same legend as Fig. 1.

not as significant as either two-way interaction ($P = .02$), it is apparent that the effects of the magnesium-phosphorus interaction tends to counteract those of magnesium-calcium interactions.

Provided the diets contained at least 0.4% phosphorus, supplemental magnesium decreased urinary phosphorus significantly, irrespective of the dietary level of calcium (Fig. 4). The analysis of variance clearly indicates a significant main effect of magnesium as well as significant magnesium-calcium and magnesium-phosphorus interactions on urinary phosphorus. Here too, the effects of these interactions counteract each other, since the three-way interaction is less than either two-way interaction.

Calcium and phosphorus balance. Supplemental magnesium made the calcium balance more positive and this is particularly evident at the higher levels of dietary calcium (Fig. 5). The analyses of variance show that there are significant two- and three-way magnesium interactions with calcium and phosphorus (Table 2).

When the diet contained at least 0.4% phosphorus, supplemental magnesium made the phosphorus balance more positive (Fig. 6) but if dietary phosphorus was low (0.2%) magnesium was without effect. The analysis of variance shows a significant main effect of magnesium on balance as well as significant interactions with calcium and phosphorus.

Serum levels of calcium and phosphorus. The analysis of variance (Table 2) indicates that magnesium had a slight main effect to lower serum calcium (Table 3). The effect of the magnesium-phosphorus interaction was to lower serum calcium at low dietary levels of phosphorus (0.2%).

With the exception of the group fed 0.2% phosphorus and 0.8% calcium, increasing the magnesium intake had no significant effect on serum phosphorus (Table 1). The interaction between magnesium and phosphorus also was found to be significant and this probably accounts for the trend towards lower levels of serum

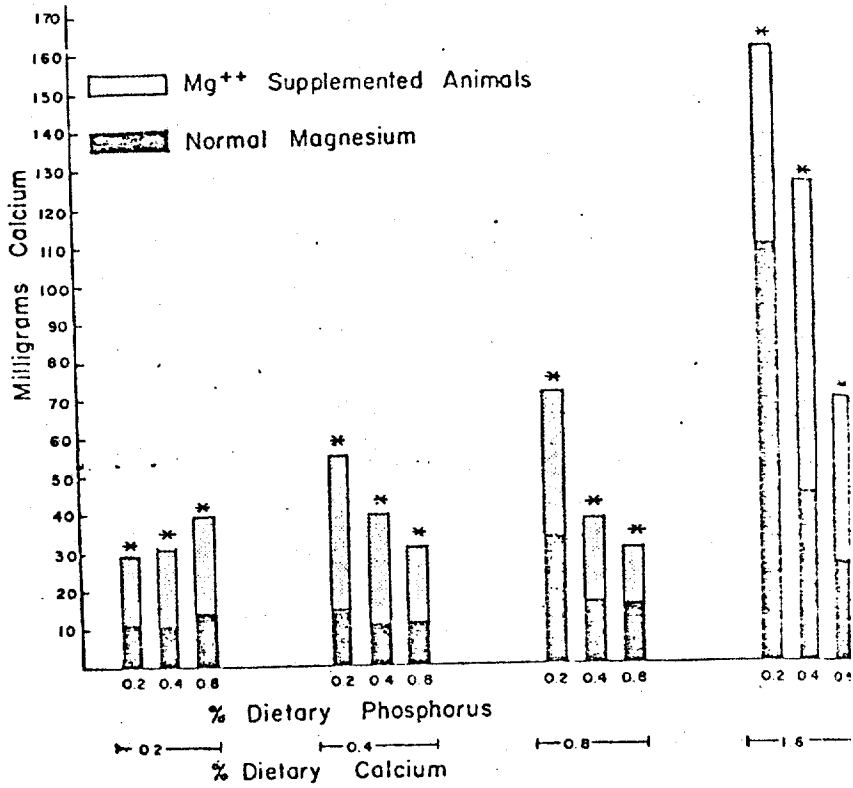


FIG. 3. Total urinary calcium—12 days. Same legend as Fig. 1.

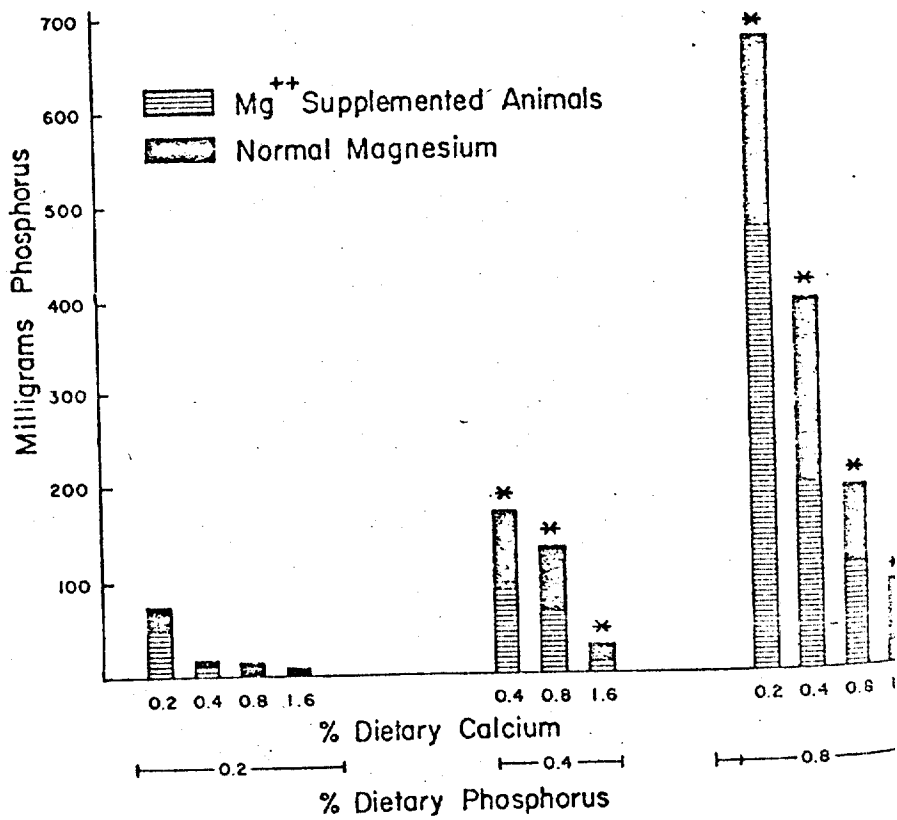


FIG. 4. Total urinary phosphorus—12 days. Same legend as Fig. 1.

phosphorus of the rats supplemented with magnesium and fed 1.6 % dietary calcium (Table 1).

DISCUSSION

An attempt has been made in these experiments to minimize the variables inherent in animal balance studies by using animals of the same sex and approximately same weight, paired food and fluid comsumption niques, semi-synthetic diets of known composition, a standard period of acclimatization to the diets pri an adequate balance period. To avoid the complic effects of rapid growth, relatively mature rats were

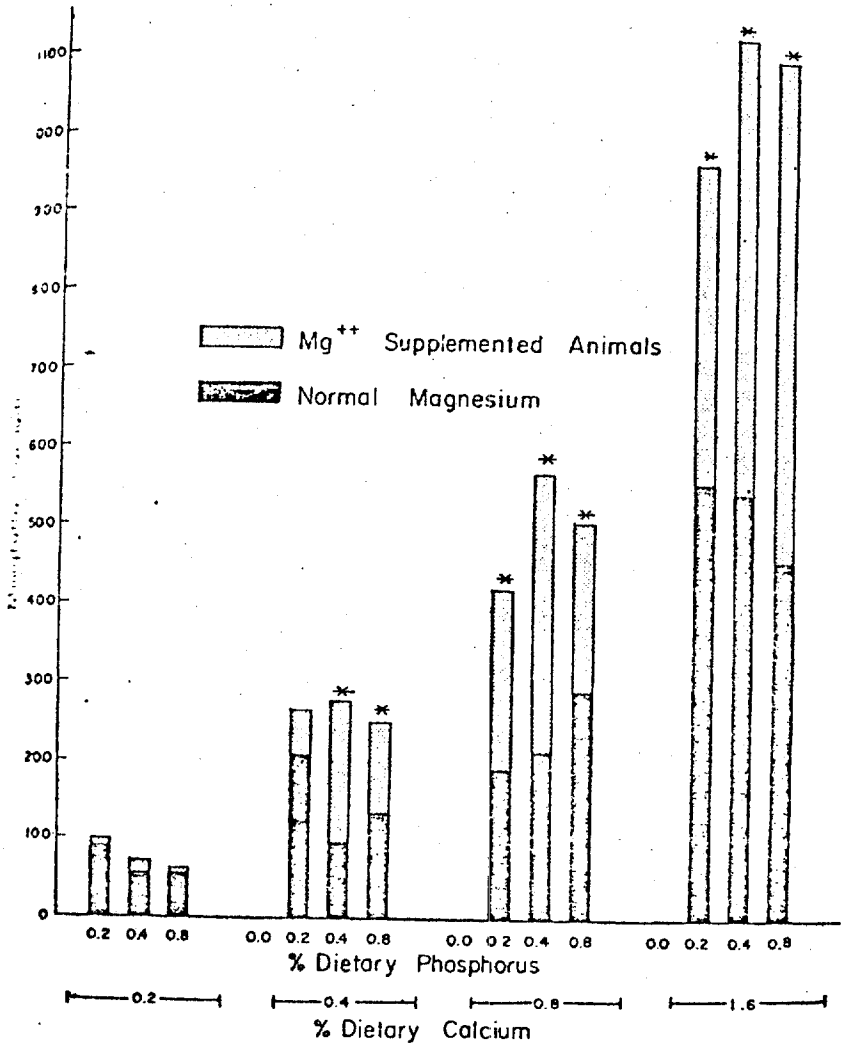


FIG. 5. Calcium balance—12 days. Same legend as Fig. 1.

As a result of the large number of animals involved, these studies were carried out over a considerable period of time and actually five different experiments comprise this study. Consequently, there would be greater variation between groups than if all experiments were carried out at one time. Despite these variations, and with one or two exceptions, the over-all effects of magnesium supplementation and dietary variations on calcium and phosphorus metabolism are very consistent. It is evident from the data that absorption and excretion of calcium and phosphorus are markedly influenced by dietary magnesium. Further, the effects of magnesium are also dependent on the dietary levels of calcium and phosphorus.

Supplemental magnesium caused many unexpected effects in calcium metabolism, and gave further evidence of its involvement in calcium homeostasis. Earlier studies (8, 10, 11) and the present experiments have shown that increased dietary magnesium decreased fecal calcium significantly, i.e., increased calcium absorption, if the diet contained at least 0.4% calcium. These findings appear to be in direct contradiction to other work which showed that, in the absence of dietary magnesium, the intestinal absorption of calcium was increased (1). The conclusions based on these latter studies were that magnesium ions inhibited the intestinal absorption of calcium. Recent studies (I. Clark and L. F. Bélanger, to be published) with increasing levels of dietary magnesium from 0.0% to 1.0% and using magnesium oxide as the supplement show that, as the dietary level of magnesium is increased, so is calcium absorption. However adult rats were used in these experiments and magnesium deficiency never was obtained. Further, in magnesium deficiency there appears to be a marked alteration in the chemical composition of the mucosal cells of the intestine (unpublished), and this perhaps may account for the increased absorption of calcium.

In vitro studies using the "everted gut sac" technique (35, 18), and the isolated intestinal loop (12) have shown that magnesium ions inhibit active transport of calcium. These studies have been quoted as supporting evidence for a common transport mechanism for calcium and magnesium in the intestine and for competition with each other in transport. However, the present in vivo studies clearly show that magnesium abets calcium absorption. Thus, it is apparent that in vitro active transport studies may not necessarily reflect the phenomenon of absorption as it occurs in vivo. Confirmation of in

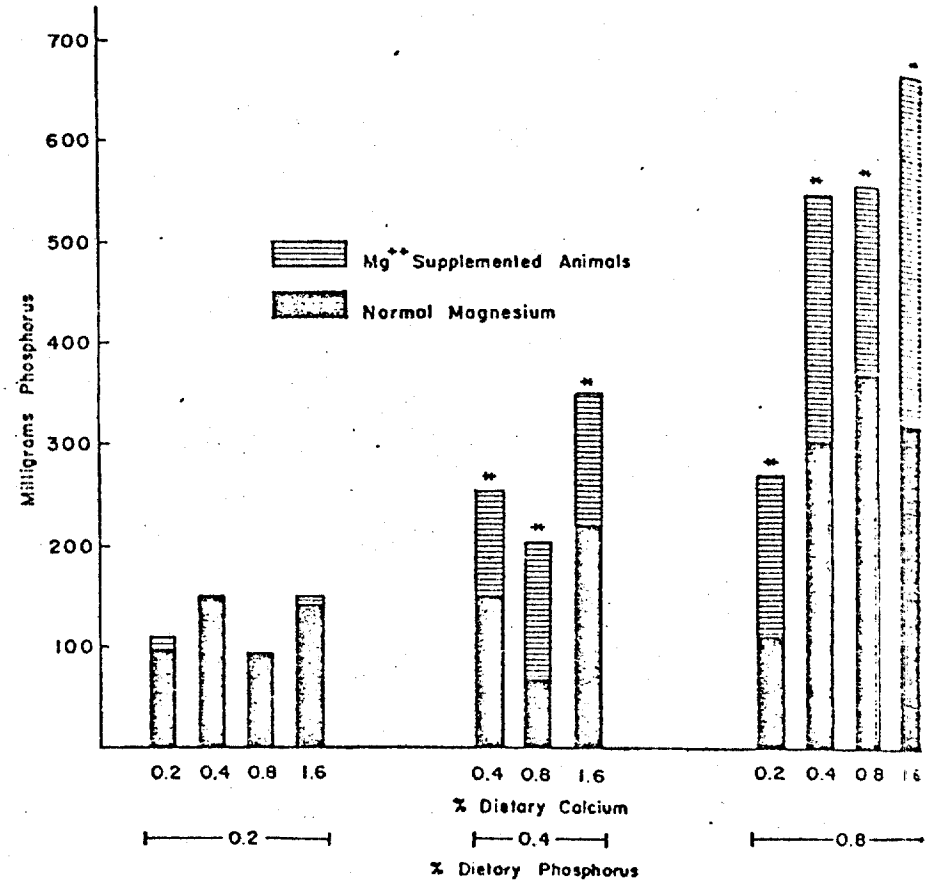


FIG. 6. Phosphorus balance—12 days. Same legend as Fig. 1.

vitro studies by in vivo studies must be made before extrapolations from active transport in the flask to intestinal absorption in the whole animal are drawn. The mechanism whereby magnesium ions increase the intestinal absorption of calcium is, at present, not known.

The increased absorption of calcium caused by supplemental magnesium is reflected in elevated urinary calcium and the question arises as to the mechanism of this hypercalciuria. It is well known that acidifying salts such as NH_4Cl (20) and acid forming diets (3) produce hypercalciuria and the question arises whether alterations in the salt content of the diet (Table 1) or the high $MgCl_2$ intake may produce such an acidifying effect. Since the salt content of the diets of the rats with and without supplemental magnesium was the same at each specific dietary level of calcium and phosphate, increased absorption of calcium and hypercalciuria cannot be attributed to any acid- or base-forming propensity of the diet. It is unlikely that acidosis produced by magnesium chloride is responsible for the hypercalciuria since acidifying salts increase urinary phosphorus and cause a negative calcium balance with loss of skeletal mass (20). In these studies, there was an increase in calcium balance as well as a significant depression in urinary phosphorus. Moreover, other magnesium salts such as magnesium oxide or magnesium hydroxide which are alkaline salts are equally effective as magnesium chloride in producing hypercalciuria (5).

Increasing calcium intake caused increased urinary calcium (Fig. 2), as has been observed previously (25, 29). The parenteral administration of magnesium also produced hypercalciuria (9, 27, 41) and, although serum magnesium was not determined in these studies, it has been shown that increasing dietary magnesium will cause elevated serum levels of magnesium (13). A depressant effect of elevated serum magnesium (26) or calcium (38) on parathyroid activity could induce hypercalciuria since decreased parathyroid activity is known to increase urinary calcium (22, 39, 40). It is unlikely that the parathyroid is the principal mechanism, since supplemental magnesium produces marked hypercalciuria even in the absence of these glands (7). Thus, the observed hypercalciuria is very likely the result of a direct action of magnesium on the kidney which either prevents the reabsorption of calcium (33) or increases the filtered load of calcium as a result of increased intestinal absorption of calcium. This latter possibility has been suggested by others (5).

In the studies reported here, it is clear that the effect of supplemental magnesium on calcium balance is dependent on the dietary level of calcium, i.e., increasing it only when the diet contained 0.4 % calcium or more. Since magnesium also increases calcium absorption and calcium balance in man (40), this element may be of importance in the treatment or prevention of certain metabolic bone diseases, particularly those resulting from inadequate calcium absorption from the intestines. It is significant that analyses of soft tissues in these experi-

TABLE 3. *Serum values, mg/100 ml*

		Percent Dietary Calcium							
		0.2		0.4		0.8		1.6	
		Calcium	Phosphorus	Calcium	Phosphorus	Calcium	Phosphorus	Calcium	Phosphorus
Percent dietary phosphorus	0.8	11.0±0.5 *11.5±1.0*	8.6±0.8 *9.3±0.9*	10.1±0.3 *9.5±1.0*	8.2±0.9 *9.7±1.0*	12.2±0.2 *12.8±1.2*	8.2±1.2 *9.4±1.7*	11.7±1.2 *12.4±0.4*	8.3±0.2 *7.7±0.9*
	0.4	12.3±0.9 *12.0±0.6*	9.0±0.6 *9.5±0.9*	10.4±0.4 *10.0±0.5*	9.0±1.0 *9.1±0.9*	13.0±0.5 *12.3±1.0*	7.3±1.7 *7.2±1.3*	12.6±0.3 *12.6±0.6*	6.7±0.4 *5.8±1.2*
	0.2	11.6±1.6 *10.7±0.7*	8.8±0.7 *8.8±0.5*	10.9±0.4 *10.8±0.3*	9.8±1.0 *8.0±0.9*	10.2±1.4 *9.2±0.8*	8.1±0.7 *5.5±0.4*	14.1±1.5 *13.2±0.9*	5.7±0.5 *5.2±0.6*

Values are means ± SD. Italicized numbers refer to the animals supplemented with magnesium.

cits, e.g., heart, kidney, muscle, and liver, showed no crease in calcium content of these soft tissues. Further, another study (I. Clark and L. F. Bélanger, to be blished) an analysis of femora has revealed an increase calcium content as well as an increase in the amount of ature bone as judged by alpharadiography with creasing amounts of dietary magnesium.

The analysis of variance indicates that magnesium, per e, has no effect on fecal phosphorus but that a magesium-calcium interaction produces a significant effect Table 2). Since magnesium increases calcium absorption from the intestine and thereby decreases the amount of calcium available for precipitation of phosphate in the intestine, the net result is to enable more phosphorus to be absorbed and less to be excreted in the stool (Fig. 2). Such an observation may explain the magnesium-calcium interaction. If, however, dietary phosphorus is adequate for the body's needs (0.2%), it has been shown that the animal will conserve as much of this element as possible (19) and magnesium, in this situation, does not affect phosphorus absorption, since maximal absorption already is occurring. This low fecal phosphorus probably represents bacterial as well as some unabsorbable dietary phosphorus (19). Also, if the dietary calcium-to-phosphorus ratio is larger, supplemental magnesium will increase fecal phosphorus (Fig. 2). This is very likely the reason why the three-way interaction is not significant (Table 2).

The analysis of variance shows that urinary phosphorus, unlike fecal phosphorus, is directly dependent not only on magnesium per se, but also on magnesium-calcium-phosphorus interactions. Again, at low levels of dietary phosphorus (0.2%), supplemental magnesium had no effect on urinary phosphorus, but decreased it significantly if the diet contained at least 0.4% phosphorus. The mechanism causing hypophosphaturia is not apparent but the parathyroid gland does not seem to be involved since hypophosphaturia also is induced by magnesium ions in aparathyroid rats (7). The fall in urinary phosphorus, caused by magnesium, may be responsible in part for the suppression and prevention of urolithiasis that has been shown to occur in renal stone-forming patients following the administration of magnesium ions (28, 34). The improvement in phosphorus balance seen with supplemental magnesium appears to be primarily the result of renal conservation of phosphorus since, in some instances, fecal excretion was unaffected.

The author extends his sincere appreciation to Mr. George P. H. Styan and Mr. Peter C. Trenholme of the Dept. of Mathematical Statistics, Columbia University, for detailed statistical analyses performed on the data.

REFERENCES

1. Alcock, N., and I. MacIntyre. Inter-relation of calcium and magnesium absorption. *Clin. Sci.* 22: 185–193, 1962.
2. Anderson, T. W. *An Introduction to Multivariate Statistical Analysis.* New York: Wiley, 1958.
3. Bogert, L. J., and E. Kirkpatrick. Studies in inorganic metabolism. II. The effects of acid-forming and base forming diets upon calcium metabolism. *J. Biol. Chem.* 54: 375–386, 1922.
4. Bogert, L. J., and E. J. McKittrick. Interrelations between calcium and magnesium metabolism. *J. Biol. Chem.* 54: 363–374, 1922.
5. Briscoe, A. M., and C. Ragan. Effect of magnesium on calcium metabolism. *Am. J. Clin. Nutr.* 19: 296–306, 1966.
6. Carwell, H. E., and J. E. Winter. The effects of high and prolonged magnesium lactate intake upon the metabolism of magnesium and calcium in man. *J. Biol. Chem.* 93: 411–418, 1931.
7. Clark, I. Agents affecting the removal of skeletal radiocalcium and radiophosphorus from intact and parathyroidectomized rats. In: *The Parathyroids,* edited by R. O. Greep and R. V. Talmage. Springfield, Ill.: Thomas, 1961, p. 193.
8. Clark, I. The relation of magnesium ions to calcium and phosphate absorption. *Nature* 207: 932, 1965.
9. Clark, I., and R. Geoffroy. Studies in calcium metabolism. *J. Biol. Chem.* 233: 203–205, 1958.
10. Clark, I., C. Moyer, and F. Rivera-Cordero. The effect of magnesium on calcium and phosphate absorption and excretion. *Federation Proc.* 24: 372, 1965.
11. Clark, I., F. Rivera-Cordero, and S. Cohn. Some criteria for the removal of skeletally bound radioactive strontium. *Proc. Soc. Exptl. Biol. Med.* 118: 879–882, 1965.
12. Cramer, C. F., and J. Dueck. *In vivo* transport of calcium from healed Thiry-Vella fistulas in dogs. *Am. J. Physiol.* 202: 161–164, 1962.

13. Cunningham, I. J. The influence of the level of dietary magnesium on the magnesium and calcium contents of the bones, the bodies, and the blood serum of rats. *New Zealand J. Sci. Technol.* 15: 191–198, 1933.
14. Givens, M. H. Studies in calcium and magnesium metabolism: Experiments in man. *J. Biol. Chem.* 34: 119–130, 1918.
15. Haag, J. R., and L. S. Palmer. The effect of variations in the proportions of calcium, magnesium and phosphorus contained in the diet. *J. Biol. Chem.* 76: 367–388, 1928.
16. Hart, E. B., and H. Steenbock. The effect of a high magnesium intake on calcium retention by swine. *J. Biol. Chem.* 14: 75–80, 1913.
17. Heaton, W. F., and E. M. Parsons. Metabolic effect of high magnesium intake. *Clin. Sci.* 21: 273–284, 1961.
18. Hendrix, J. Z., N. W. Alcock, and R. M. Archibald. Competition between calcium, strontium and magnesium for absorption in the isolated rat intestine. *Clin. Chem.* 9: 734–744, 1963.
19. Henry, K. M., and S. K. Kon. Studies on the metabolism of calcium and phosphorus and on the availability of these elements from milk and from an inorganic source. *Biochem. J.* 33: 173–191, 1939.
20. Jaffe, H. L., A. Bodansky, and J. P. Chandler. Ammonium chloride decalcification, as modified by calcium intake; relation between generalized osteoporosis and ostitis fibrosa. *J. Exptl. Med.* 56: 823–834, 1932.
21. King, E. J. In: *Micro-analysis in Medical Biochemistry.* London: Churchill, 1951, p. 68.
22. Kleeman, S. R., R. E. Rockney, and M. H. Maxwell. The effect of parathyroid extract (PTE) on the renal clearance of diffusible calcium. *J. Clin. Invest.* 37: 907, 1958.
23. Knapp, E. L. Factors influencing the urinary excretion of calcium. I. In Normal Persons. *J. Clin. Invest.* 26: 182–202, 1946.
24. Kruse, H. D., E. R. Orent, and E. V. McCollum. Studies on magnesium deficient animals. *J. Biol. Chem.* 66: 519–539, 1932.
25. Loken, H. F., J. S. Teal, and E. Eisenberg. Flame spectrophotometry of calcium with reversed oxacetylene flame. Applications to serum and urine. *Anal. Chem.* 35: 875–880, 1963.
26. MacIntyre, I., S. Biss and V. A. Troughton. Parathyroid hormone and magnesium homeostasis. *Nature* 198: 1058–1060, 1963.
27. Mendel, L. B., and S. R. Benedict. The paths of excretion for inorganic compounds. I–IV. The excretion of magnesium. *Am. J. Physiol.* 25: 1–22, 1909.
28. Moore, C. A., and G. E. Bunce. Reduction in frequency renal calculus formation by oral magnesium administratio *Invest. Urol.* 2: 7–13, 1964.
29. Nicolaysen, R., N. Eeg-Larsen, and O. J. Malm. Physiolo of calcium metabolism. *Physiol. Rev.* 33: 424–444, 1953.
30. O'Dell, B. L. Magnesium requirement and its relation other dietary constituents. *Federation Proc.* 19: 648–654, 19
31. O'Dell, B. L., E. R. Morris, E. E. Pickett, and A. Hogan. Diet composition and mineral balance in guinea pi *J. Nutr.* 63: 65–77, 1957.
32. O'Dell, B. L., E. R. Morris, and W. O. Regan. Magnesiu requirement of guinea pigs and rats. Effect of calcium a phosphorus and symptoms of magnesium deficiency. *J. Nu* 70: 103–111, 1960.
33. Samiy, A. H., J. L. Brown, D. I. Globus, R. H. Kessle and D. D. Thompson. Interrelations between renal transpo systems of magnesium and calcium. *Am. J. Physiol.* 19 599–602, 1960.
34. Sauberlich, H. E., G. E. Bunce, C. A. Moore, and O. Stonington. Oral magnesium in the treatment of ren calculus formation. *Am. J. Clin. Nutr.* 14: 240, 1964.
35. Schachter, D., and S. M. Rosen. Active transport of C by the small intestine and its dependence on vitamin D. *Am. Physiol.* 196: 357–362, 1959.
36. Scheffé, H. *The Analysis of Variance.* New York: Wiley, 195
37. Sjollema, B. Nutritional and metabolic disorder in catt *Nutr. Abstr. Rev.* 1: 621–632, 1932.
38. Stoerk, H. C., and W. H. Carnes. The relation of the dieta Ca:P ratio to serum Ca and to parathyroid volume. *J. Nu* 29: 43–50, 1945.
39. Talmage, R. V. Studies on the maintenance of serum calciu levels by parathyroid action on bone and kidney. *Ann. N York Acad. Sci.* 64: 326–335, 1956.
40. Talmage, R. V., and F. W. Kraintz. Progressive changes renal phosphate and calcium excretion in rats followi parathyroidectomy or parathyroid administration. *Proc. S Exptl. Biol. and Med.* 87: 263–267, 1954.
41. Tibbetts, D. M., and J. C. Aub. Magnesium metabolism health and disease. I. The magnesium and calcium excreti of normal individuals, also the effect of magnesium, chlori and phosphate ions. *J. Clin. Invest.* 16: 491–501, 1937.
42. Tufts, E. V., and D. M. Greenberg. The biochemistry magnesium deficiency: chemical changes from magnesiu deprivation. *J. Biol. Chem.* 122: 693–714, 1938.
43. Welt, L. G. Experimental magnesium depletion. *Yale J. Bi Med.* 36: 325–349, 1964.

FATAL MAGNESIUM POISONING

*Following Magnesium Sulfate, Glycerin, and Water Enema in Primary Megacolon**

E. N. COLLINS, M.D., and P. W. RUSSELL, M.D.
Department of Gastroenterology

THE inclusion of magnesium sulfate in an enema in the presence of megacolon is extremely dangerous. Magnesium sulfate is unique in that it has both cathartic and anticonvulsant properties. When used to prevent convulsions it is given parenterally. When used as a cathartic, orally, in the presence of intestinal disease, the permeability of the intestinal membrane to magnesium may be increased and the effects on nerve function may predominate. The fact that the use of Epsom salt as a cathartic and in the treatment of biliary tract disease is usually harmless may lead to overlooking the possibility of its poisonous effects due to untoward absorption.

In the case reported death was due to magnesium poisoning resulting from absorption from an enema consisting of magnesium sulfate, glycerin, and water. An autopsy was performed.

Case Report

A boy, aged 4, was admitted to the Cleveland Clinic Hospital on July 2, 1948 with a history of abdominal enlargement and constipation present since birth. The patient never had had a spontaneous bowel movement. He had taken mineral oil orally each day since birth, but had no bowel function unless he was given pint enemas of mineral oil or soapsuds 1 to 4 times daily in addition. A left retroperitoneal ganglionectomy had been performed in 1946, and anal dilatation in 1947, without alteration of bowel habit. He had vomited about once a month.

Physical examination revealed an undersized boy of 4 years, weighing 25 pounds, with symmetrical abdominal enlargement. The abdominal wall was slightly atrophic and of doughy consistency. There was a tympanitic note to percussion in all quadrants, and no organs or masses were palpable. Hypoperistalsis was evident on auscultation. Digital rectal examination revealed a relaxed sphincter and a dilated rectum. The remainder of the physical examination did not reveal abnormalities.

Examination of the blood disclosed 11.5 Gm. of hemoglobin per 100 cc., 5,870,000 erythrocytes, and 11,000 leukocytes. The fasting blood sugar was 66 mg. per 100 cc. The urinalysis, blood Wassermann and Kahn tests were negative. A barium enema revealed filling of the colon to the splenic flexure, with a striking dilatation of the rectum and sigmoid. Further filling of the colon was unnecessary, as an extensive accumulation of gas demonstrated dilatation of the colon in all segments (fig. 1).

The diagnosis of primary megacolon was made. To determine the possible effect of lumbar sympathectomy in compensating for the neuromuscular dysfunction of the colon, a lumbar subarachnoid injection of 75 mg. of procaine was performed on July 8. Despite

*Dr. A. C. Corcoran, Research Division, assisted in the analysis of the factors involved in this case.

*Dr. Edith Farnsworth, Northwestern University School of Medicine, kindly performed the analysis which is gratefully acknowledged.

adequate level of anesthesia, spontaneous evacuation of the bowel did not occur. On July 9, at 2 p.m., a caudal epidural injection of procaine was given, which produced relaxation of the anal sphincter and increased peristalsis in the small bowel, but did not result in the passage of feces. After complete recovery from the anesthetic, at 3:30 p.m. an enema was administered. Prostigmine was to have been given to cause evacuation. The enema consisted of 2 ounces of a solution of magnesium sulfate containing 30 Gm. of the hydrated salt, 4 ounces of glycerin, and 6 ounces of water. Without expelling the enema, the child "fell asleep" about 4 p.m. At 5 p.m. he was found to be comatose, with complete flaccid paralysis

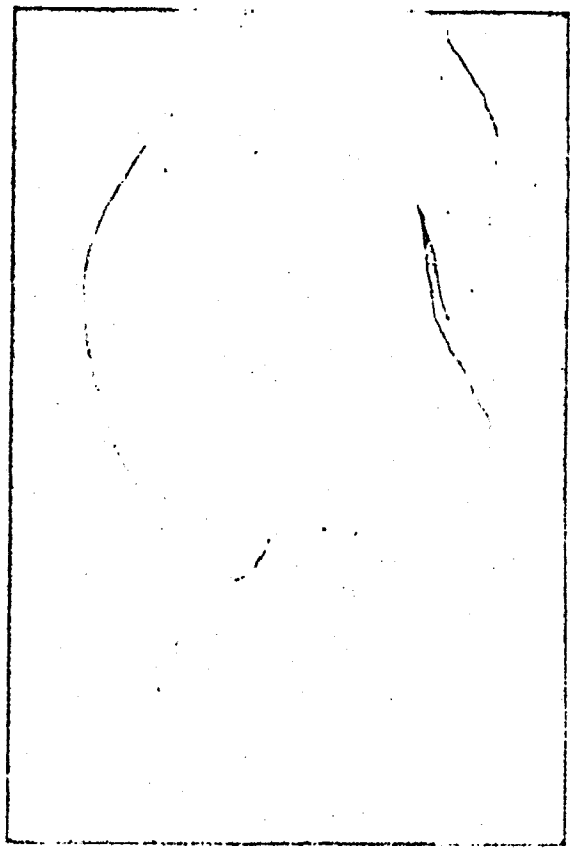


FIG. 1. Barium enema showing sigmoid and part of descending colon.

and absent pupillary reflexes. The respiratory rate was 8 per minute. This is important and characteristic. The pulse rate was 116 per minute. Cyanosis was absent. The patient was estimated to be in the second plane of third stage anesthesia. A rectal tube was immediately inserted. A subcutaneous injection of 0.4 cc. of prostigmine methylsulfate 1:5000 was given and caused release of about 1 pint of liquid fecal material. Following artificial respiration, oxygen, and 0.2 cc. of nikethamide subcutaneously the respiratory rate increased gradually to 16 per minute. The sequence of events indicated that the coma was related to the retained enema and probably to its magnesium content. Accordingly, at 6:30 p.m., 2 cc. of a 10 per cent solution of calcium gluconate was injected into the jugular vein, and an equal dose was

146

tramuscularly. Shortly thereafter, a slight increase in the respiratory rate was the neurological picture was unchanged. Despite continued supportive measures, aspiration of additional fecal material, injections of 0.3 cc. of nikethamide, and caffeine with sodium benzoate, the patient's condition remained the same until respiratory paralysis at 10:30 p.m.

ortem examination showed the peritoneal cavity to be free of fluid and adhesions, was hypertrophied, elongated, and grossly dilated, measuring 10 cm. in diameter. tions or stercoral ulcerations were found. There was a considerable quantity of ial in the left colon, but the small intestines were empty. A Meckel's diverticulum ated. There was no evidence of intestinal obstruction. Microscopic examination on revealed thin mucosa infiltrated with leukocytes, an increased amount of l connective tissue, and thickening of the muscular coats and serosa. Other microscopic findings included severe cerebral congestion with slight edema, of the liver, lungs, and spleen, edema of the myocardium, and vacuolization of l cortex. A pathologic diagnosis of primary megacolon was made by Dr. John B. nasmuch as the gross and microscopic examination failed to reveal the cause of ecimen of heart blood was analyzed for magnesium.*

erum magnesium at autopsy was 30 mg. per 100 cc. (25 mEq per liter), and the ium was 20.8 mg. per 100 cc.

Discussion

known that progressive increase in the serum magnesium level above nal of 2.4 mg. per 100 cc. results in nervous depression. In the cat anges are apparently due to blocked neuromuscular transmission ffects different systems as the magnesium level rises:[1,2] tendon reflexes ar at 6-14 mEq per liter, coma occurs at about 14.2 mEq per liter, respiration disappears at 15-20 mEq per liter, and corneal reflexes 5 mEq per liter. All parts of the cardiac conduction system are also , S-A and A-V block occurring above a level of 15 mEq per liter. arrest never precedes respiratory arrest, and usually occurs at levels 4 mEq per liter. It may be concluded, therefore, that death in this s due to respiratory paralysis.

10 cases included in separate reports by Fräser[3] in 1909 and Boos[4] in nly 2 presented the symptoms of coma and flaccid paralysis which ite the known effects of magnesium poisoning; others manifested shock, , or "intestinal intoxication" due to the local effects of overdosage. ll[5] in 1929 reported the rapid onset of coma, paralysis, and death due oneal absorption of an enema containing 15 Gm. of magnesium sulfate. d been given to a patient whose sigmoid colon had been inadvertently ted. Byron[6] in 1939 reported 5 cases of fatal poisoning following oral tration of Epsom salt to children with helminthiasis. An unpublished following rapid intravenous injection of 7.5 Gm. of magnesium sulfate by Winkler, et al.[7] in 1942. In none of these 9 typical cases of mag- poisoning, all of which were fatal, were there confirmatory laboratory

weett and Gens[8] in 1943 reported 2 cases of magnesium poisoning follow- emas, one presumptive, and the other the *first proved case*. One patient, ar old boy, had an imperforate anus which had been treated with colos-

tomy followed by an abdominoperineal plastic operation. Two months post-operatively, an enema containing magnesium immediately resulted in flaccid paralysis, coma, and absent pupillary reflexes. Respiratory failure occurred a few hours later, and autopsy failed to reveal the cause of death. A 23 year old woman with ureteral calculus was given prostigmine and an enema containing 28 Gm. of magnesium sulfate. One hour later, she was found to be in a coma, with flaccid paralysis and sluggish pupils. One gram of calcium gluconate intravenously gave prompt improvement. Three hours later, the serum magnesium was 20.8 mg. per 100 cc., and the serum calcium 9.9 mg. per 100 cc. An additional 1 Gm. of calcium gluconate intravenously resulted in complete recovery of consciousness, at which time the serum magnesium was 15.4 mg. per 100 cc., and the serum calcium 11.2 mg. per 100 cc. Of the 11 reported typical cases of magnesium poisoning, this was the only one to recover.

A fact not sufficiently appreciated is that the gastrointestinal membrane is by no means impermeable to the salts of magnesium. Hirschfelder[9] found that 40 per cent of an oral dose of magnesium sulfate was absorbed within twenty-four hours; he emphasized that the rapid urinary excretion of magnesium which normally prevails prevented a significant rise in serum concentration, and that when excretion was depressed, as in nephritis, dangerous serum magnesium levels could be obtained with an ordinary oral dose. Van Liere[10] demonstrated in dogs 14 per cent absorption of magnesium in ninety minutes from ileal loops filled with 3.25 per cent anhydrous magnesium sulfate.

Winkler, et al. calculated the fraction of the body water in which magnesium is distributed following a known retention of an injection of magnesium sulfate. Assuming from their data that the increased magnesium concentration prevailed in 30 per cent of the body weight of our patient, it appears that this child absorbed at least 34 per cent of the 3000 mg. of magnesium present in the enema. The reason for this absorption was not evident from the postmortem examination. Other cases of primary megacolon are known to have received such enemas without untoward results; Bonar[11] repeatedly administered enemas containing 56 Gm. of magnesium sulfate to a 4 year old child with megacolon and obtained excellent evacuations of the bowel.

The only effective antidote in magnesium poisoning is parenteral administration of calcium ion. The proper dosage of calcium needed to antagonize a given magnesium excess has not been calculated. The response to calcium obtained in experimental animals appears immediately so that the dosage can best be judged by clinical improvement. This was also apparent in the case presented by Fawcett and Gens, who injected 90 mg. of calcium ion. The dose of 36 mg. of calcium ion used in our case would seem sufficient by comparing body weights, yet no clinical improvement was noted.

Curiously, the final serum calcium level was 20.8 mg. per 100 cc. This hypercalcemia is difficult to explain. Assuming a plasma volume of 570 cc., the total increase in serum calcium was about 57 mg., of which 36 mg. were injected, leaving an excess of at least 21 mg. of calcium in the serum. This might be regarded as a protective response of the body to hypermagnesemia.

We have not found this effect to be mentioned in the literature. That this hypercalcemia might also have blocked neuromuscular transmission seems unlikely, as higher levels of serum calcium are needed for such a depression to occur. We are, therefore, led to assume that the lack of response to calcium in this case was due not to under-dosage but to delay in its administration.

Summary

1. Fatal magnesium poisoning resulted from the absorption from an enema in a patient with primary megacolon.

2. The clinical signs of flaccid paralysis, slow respiratory rate, coma, and absent pupillary reflexes developing rapidly after the administration of magnesium sulfate must be promptly recognized if calcium therapy is to be beneficial.

3. The use of magnesium sulfate enemas is contraindicated in children with primary megacolon.

References

1. Hoff, H. E., Smith, P. K., and Winkler, A. W.: Effects of magnesium on nervous system in relation to its concentration in serum. Am. J. Physiol. 130:292-297 (Aug.) 1940.
2. Smith, P. K., Winkler, A. W., and Hoff, H. E.: Electrocardiographic changes and concentration of magnesium in serum following intravenous injection of magnesium salts. Am. J. Physiol. 126:720-730 (July) 1939.
3. Fraser, C.: Epsom salts as a poison: with a record of a case of unusual symptoms due to this drug. Lancet 1:1174-1176, 1909.
4. Boos, W. F.: Magnesium poisoning. J.A.M.A. 55:2037-2042 (Dec. 10) 1910.
5. Maxwell, W. W.: Traumatic perforation of uterus with severance of rectum; report of a case. Am. J. Obst. and Gynec. 17:96-102 (Jan.) 1929.
6. Byron, F. E.: Fatal results following administration of magnesium sulfate. J. Malaya Br., Brit. M. A. 3:100-101 (June) 1939.
7. Winkler, A. W., Smith, P. K., and Hoff, H. E.: Intravenous magnesium sulfate in treatments of nephritic convulsions in adults. J. Clin. Investigation 21:207-216 (March) 1942.
8. Fawcett, D. W., and Gens, J. P.: Magnesium poisoning following enema of epsom salt solution. J.A.M.A. 123:1028-1029 (Dec. 18) 1943.
9. Hirschfelder, A. D.: Clinical manifestations of high and low plasma magnesium; dangers of epsom salt purgation in nephritis. J.A.M.A. 102:1138-1141 (April 7) 1934.
10. Van Liere, E. J., Northrup, D. W., Stickney, J. C., and Richards, R. E.: Effect of anemia anoxia on absorption of isotonic magnesium sulfate from small intestine. Proc. Soc. Exper. Biol. and Med. 64:62-64 (Jan.) 1947.
11. Bonar, B. E.: Pelvirectal acholasia (Hirschsprung's disease); temporary medical management by rectal administration of magnesium sulfate. Am. J. Dis. Child. 48:123-129 (July) 1934.

French Translation

Burns followed by Erythmato-Pigmented lesions due to Harsh Resuscitation with Epsom salt; Colomb, D., Perrot, H., and Beyvin, A.J.

Bull. Soc. Fr. Derm. Syph. 76: 133 (1969)

Mr. M. Michel, 25 years old became ill at the movies on April 31, 1968. An emergency aid present in the room placed a bottle of smelling salts under the nostrils of the stretched-out sick man. Part of the contents spilled on his face and in his mouth causing caustic lesions.

Thirty-six hours later, examination showed: blistered erythmatic lesions on the lips and on the internal surface of the left cheek, involving a dysphasia and discomfort at the mouth opening; on the other side of the mouth, an erythmatic-pigmented trail descended to the cervical artery region with an outline of loosening blisters. A soothing and disinfecting local treatment was prescribed. Two days later the intrabuccal lesions were improved but the lesions on the lips and face were infected and the erythmatic channels had a strong tendency to pigmentation. General antibiotic therapy was prescribed. By the 11th of May, the scars were healed, but the pigmented tracks crossing the cheeks created a serious detrimental esthetic effect. On May 19, 29 days after the accident, the pigmentation had regressed considerably.

The case history of this illness focuses attention on the caustic qualities of certain liquids normally used for inhalation for the purpose of resuscitation. The product used was a solution of smelling salts no.

132.521, whose manufacturer or composition we have been unable to ascertain. The pigmentation of the skin lesions in channels, recalls those caused by certain perfumes, but their evolution was quite quickly favorable.

p. 133

10

Brûlures suivies de lésions érythémato-pigmentées par réanimation brutale avec des « sels anglais »,

par MM. D. Colomb, H. Perrot et A.-J. Beyvin.

M. Mol... Michel, âgé de 25 ans, a présenté un malaise dans une salle de cinéma le 31 avril 1968. Un secouriste présent dans la salle plaça sous les narines du malade allongé une bouteille de « sels anglais » dont une partie du contenu coula sur le visage et dans la bouche, provoquant des lésions caustiques.

L'examen constatait 36 heures plus tard : des lésions érythémato-bulleuses des lèvres, de la face interne de la joue gauche, entrainant une dysphagie et une gêne à l'ouverture de la bouche: de part et d'autre de la bouche : des traînées érythémato-pigmentées descendant jusqu'à la région cervicale avec ébauche de décollements bulleux. Un traitement local adoucissant et désinfectant fut prescrit. Deux jours plus tard les lésions intrabuccales étaient améliorées, mais les lésions des lèvres et du visage étaient surinfectées et les coulées érythémateuses avaient une forte tendance à la pigmentation: une antibiothérapie générale fut prescrite. Le 11 mai la cicatrisation était complète, mais des coulées pigmentées barrant les joues créaient un préjudice esthétique important. Le 19 mai, 20 jours après l'accident, la pigmentation avait régressé considérablement.

L'histoire de ce malade met l'accent sur la causticité de certains liquides utilisés normalement en inhalation en vue d'une réanimation. Le produit utilisé était une solution de « sels anglais n° 132.521 » dont nous n'avons pu connaître ni le fabricant, ni la composition. La pigmentation des lésions cutanées en coulées rappelait celle provoquée par certains parfums, mais l'évolution en a été assez rapidement favorable.

Bull. Soc. Fr. Derm. Syph
76: 133 (1969)

J Nutrition, 79: 407- 15 (1963)

Excretion of Sodium, Potassium, Magnesium and Iron in Human Sweat and the Relation of Each to Balance and Requirements

C. FRANK CONSOLAZIO, LEROY O. MATOUSH, RICHARD A. NELSON, RICHARD S. HARDING AND JOHN E. CANHAM
U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado

In two recent studies on calcium (Consolazio et al., '62a) and nitrogen (Consolazio et al., '62b) excretions in sweat, it was observed that sweat losses were fairly high, accounting for 18 to 30% of the total calcium excretion and 13 to 18% of the total nitrogen excreted, of men exposed to 100°F environmental temperatures. As a result of these fairly high excretions of calcium and nitrogen in sweat, other mineral contents were also investigated in relation to intake, balance and requirements. These minerals included sodium, potassium, magnesium, phosphorus and iron. In addition, the daily osmolarity was also computed from the freezing point of the sweat.

A considerable amount of data is available in the literature on the concentration of minerals in sweat, but very few, if any, of these studies have been related to balance and requirements. Many values for the sodium, potassium, magnesium, iron and phosphorous concentrations in sweat have been thoroughly reviewed by Schwartz ('60) and Altman and Dittmer ('61). Sodium values ranged from 13 to 1[illegible] mEq/liter of sweat (30 to 240 mg/100 ml), potassium from 2.3 to 16.0 mEq/liter (9 to 62 mg/100 ml), magnesium from 0.04 to 4.5 mg/100 ml, iron from 0 to 5.3 mg/hour, and phosphorus from 0 to 43 µg/100 ml of sweat.

The fairly high nitrogen and calcium excretions in sweat reported previously (Consolazio et al., '62a,b), especially during profuse sweating conditions, indicate that mineral losses in sweat may be sufficiently important to warrant further investigation. It is the opinion of investigators (Mitchell and Hamilton, '49; Mitchell and Edman, '62) that sweat losses should be included as part of the total daily output in balance studies, otherwise erroneous conclusions can be drawn.

An attempt was made to design a study to answer some of the following questions: (a) what are the mineral losses in relation to high sweat rates; (b) what are the mineral losses in relation to the daily intake and their effect on balance studies; (c) what are the mineral losses in relation to mineral allowances and requirements; and (d) do these sweat losses decrease after acclimatization?

EXPERIMENTAL DESIGN

A study was conducted for a total of 32 days on three healthy young men. It consisted on an 8-day preliminary period (1) at 24°C (75°F) and 50% relative humidity (RH), four 4-day periods at 37.8°C (100°F) and 70% RH (1, 3, 4 and 5), and an 8-day recovery or adjustment period at 24°C (75°F) and 70% RH (6), and sweat rates were computed daily for each man. Sweat samples were collected during morning and afternoon periods. Since the quantities of sweat collected during the control and adjustment periods at 75°F were limited, no values will be reported in this paper. The daily physical activity was constant and consisted of only 30 minutes of moderate activity on the bicycle (ergometer), the remainder of the day being spent in sedentary type activities. When the men left the test area (evening) no moderate to heavy activities were permitted, the test subjects being closely supervised by the Metabolic Ward personnel, during these hours.

Received for publication October 3, 1962.

All of the test phases were performed in an environmental chamber from 8:00 AM to 11:30 AM and from 12:30 PM to 4:30 PM daily with the exception of each fourth evening when the men slept overnight in the hot room at 100°F. At this time sweat samples were collected for 2- to 5-hour intervals to determine whether the sweat concentrations of the minerals were decreased with longer exposure to the heat.

The menus consisted of 4 diets that were rotated during each period. The diets supplied 8.7 gm of sodium, 2.49 gm of potassium, 343 mg of magnesium, 23.4 mg of iron and 1.41 gm of phosphorus per day. All food was offered and consumed at regular mealtimes and no other food was permitted during the day. Salt was the only mineral available ad libitum and was measured for each man during each period.

Chemical analyses of the food composites, urine, feces and sweat were performed for sodium, potassium (Baird, '53), magnesium, iron, and phosphorus (Consolazio and Johnson, '60). In addition freezing points (Fiske, '54) were performed on the daily sweats.

Sweat rates were measured for each period using the method of Adolph et al. ('47). These sweat rates were determined by measuring weight changes during the morning and afternoon periods and then adjusting for water intake, and for water loss in urine and feces. (Gaseous exchanges of respiration contributes a small quantity, which usually consitutes less than 1% of the observed decrease in body weight.) Sweat samples were collected daily during both the morning and afternoon periods for a total of 7.5 hours. These samples were collected in polyethylene bags which covered one whole ar[illegible] and were representative for the en[illegible] period each subject was in the cham[illegible] The arm and the bag were rinsed with [illegible] tilled water and dried before each co[illegible] tion period began.

Complete urine and fecal collecti[illegible] were made for 4-day periods coincid[illegible] with the repetition of the 4 diets utili[illegible] Mineral balances were computed on e[illegible] man for each period, based on the in[illegible] sion and exclusion of the minerals [illegible] sweat as a source of mineral loss.

RESULTS

The chemical analyses of the vari[illegible] minerals performed on the cell free sw[illegible] are presented in quantities/100 ml [illegible] sweat (table 1). This table also inclu[illegible] the means and standard deviations dur[illegible] the 7.5 hours' exposure to 100°F temp[illegible] tures. After the first 4 days of acclimat[illegible] tion to heat, the potassium excretion [illegible] sweat ranged from 25 to 28 mg/100 [illegible] magnesium excretion ranged from 0.61 [illegible] 0.64 mg/100 ml, iron from 25 to 39 [illegible] 100 ml, and phosphorus from 0.11 to 0[illegible] mg/100 ml of sweat. The mean excret[illegible] rate in milligrams per hour for the sa[illegible] exposure periods ranged from 78–106 [illegible] for potassium, 2.0–2.4 mg for magnesiu[illegible] 0.11–0.16 mg iron, and 0.45–0.81 m[illegible] hour for phosphorus. The sodium exc[illegible] tion in sweat was quite variable due to t[illegible] fact that salt intake was not contro[illegible] (table 1). The daily 7.5-hour mineral [illegible] cretion and total osmolarity in sweat [illegible] 16 consecutive days at 100°F are co[illegible] pared in table 2.

On three separate occasions when t[illegible] men spent 24 hours continuously at 100 [illegible] it was observed that the sweat rate and t[illegible] sodium, potassium and iron excretions [illegible]

TABLE 1

Concentration of minerals in sweat;[1] mean for three men for 16 days of exposure at 100°F (1961 stu[illegible]

Minerals	Days' exposure			
	1–4	5–8	9–12	13–16
Sodium, mg/100 ml	337 ± 183.0[2]	113 ± 67.5	118 ± 99.4	420 ± 296.3
Potassium, mg/100 ml	84 ± 72.1	25 ± 12.6	25 ± 6.3	28 ± 9.7
Magnesium, mg/100 ml	0.70 ± 0.29	0.61 ± 0.21	0.61 ± 0.16	0.64 ± 0.[illegible]
Iron, μg/100 ml	36.4 ± 21.2	33.2 ± 16.0	38.6 ± 21.1	25.0 ± 20.9
Phosphorus, mg/100 ml	0.155 ± 0.119	0.260 ± 0.152	0.109 ± 0.087	— —

[1] Concentration/100 ml of sweat as collected and centrifuged. Chemical analysis on supernatant [illegible]
[2] Mean ± SD.

TABLE 2

Sweat excretion of minerals and total osmolarity; mean daily excretion of three men during daily 7.5-hour exposures at 100°F temperatures

Days of exposure 100°F	Sodium	Potassium	Magnesium	Iron	Phosphorus	Osmolarity[1]
	mg	*mg*	*mg*	*mg*	*mg*	*milliosmoles*
1	11176	2780	—	0.91	21.9	—
2	6210	3068	15.9	0.93	3.3	—
3	3535	658	18.5	1.13	3.8	194
4	—	—	—	—	—	221
5	2500	537	13.7	1.12	6.9	139
6	2379	555	15.4	0.81	5.4	131
7	—	—	—	1.12	—	143
8	4696	672	16.6	0.79	6.0	151
9	2623	660	17.3	0.76	3.1	112
10	3050	669	18.3	1.16	3.6	111
11	1644	516	18.6	1.26	5.2	111
12	4050	560	16.4	1.09	1.7	128
13	7090	772	17.5	0.83	—	120
14	7650	793	18.3	0.66	—	115
15	3380	757	18.6	1.41	—	135
16	6310	863	16.7	0.55	—	139

[1] −1.86°C for each 1000 milliosmoles.

TABLE 3

Diurnal variations of minerals in sweat, excretion per hour; mean for three men (1961 study)

Days at 100°F	Sodium			Potassium			Magnesium			Iron		
	7–8	11–12	14–15	7–8	11–12	14–15	7–8	11–12	14–15	7–8	11–12	14–15
hours[1]	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*μg*	*μg*	*μg*
0–3.5	375	234	944	57	74	108	3.2	2.3	2.8	159	107	179
15–8.5	844	289	1837	111	65	104	2.0	2.1	2.2	140	221	195
15–15	1235	1590	1331	115	101	82	2.2	1.8	1.5	110	155	84
15–23 (sleeping)	441	582	262	95	52	59	—	—	—	141	71	52
0–3.5 (next day)	626	945	841	90	85	115	1.8	2.2	2.2	101	193	73

[1] Zero time was 8 AM daily.

TABLE 4

Sodium balance, with and without sweat losses; mean for three men for 16 consecutive days (1961 study)

Days at 100°F	Intake	Output			Balance		Sweat as % of total output
		Urine	Feces	Sweat[1]	Sweat excluded	Sweat included	
	mg/day	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	
1–4	10229	2600	105	5784	+7524	+1740	68.1
5–8	8729	2170	81	3188	+6478	+3290	58.5
9–12	8729	2360	95	2840	+6274	+3434	53.7
13–16	—[2]	2440	94	6110	—	—	—

[1] Includes only sodium excreted in sweat while in environmental chamber for 7.5-hour period. During three overnight exposures to 100°F the sodium excreted in sweat averaged 902 mg/hour or 14.88 gm/the 16.5-hour period.
[2] Extra salt from shaker not recorded.

sweat were decreased by approximately 50% during the sleeping hours. On arising and being active again, the morning sweat mineral losses were again at a higher level (table 3).

Mineral balances, during the 7.5-hour collection period, were computed for sodium, potassium, magnesium, iron and phosphorus. These balances were calculated with and without the inclusion of

TABLE 5

Potassium balance, with and without sweat losses; mean for three men for 16 consecutive days (1961 study)

Days at 100°F	Intake	Output			Balance		Sweat as % of total output
		Urine	Feces	Sweat[1]	Sweat excluded	Sweat included	
	mg/day	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	
1–4	2493	2150	89	1776	+254	−1522	44.3
5–8	2493	2400	83	588	+10	−578	19.1
9–12	2493	1860	103	596	+530	−66	23.4
13–16	2493	1820	72	796	+601	−195	33.5

[1] Includes only potassium excreted in sweat for 7.5 hours in environmental chamber. During the three overnight exposures to 100°F, the potassium excreted in sweat averaged 84 mg/hour for a total of 1.39 gm for the 16.5-hour period.

TABLE 6

Magnesium balance, with and without sweat losses; mean for three men for 16 consecutive days (1961 study)

Days at 100°F	Intake	Output			Balance		Sweat as % of total output
		Urine	Feces	Sweat[1]	Sweat excluded	Sweat included	
	mg/day	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	
1–4	343	25.7	107	17.2	+210.3	+193.1	11.5
5–8	343	23.6	112	15.2	+207.4	+192.2	10.1
9–12	343	23.1	102	17.7	+217.9	+200.2	12.5
13–16	343	21.9	76	17.8	+245.1	227.3	15.4

[1] Includes only magnesium excreted in sweat for 7.5 hours in environmental chamber. During the three overnight exposures at 100°F, the magnesium excreted in sweat averaged 1.82 mg/hour for a total of 29.7 mg during the 16.5-hour period.

TABLE 7

Iron balance, with and without sweat losses; mean for three men for 16 consecutive days (1961 study)

Days at 100°F	Intake	Output			Balance		Sweat as % of total output
		Urine[1]	Feces	Sweat[2]	Sweat excluded	Sweat included	
	mg/day	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	
1–4	23.4		20.5	1.01	+2.9	+1.9	4.7
5–8	23.4		19.4	0.96	+4.0	+3.0	4.7
9–12	23.4		21.1	1.07	+2.3	+1.2	4.8
13–16	23.4		21.5	0.86	+1.9	+1.0	3.9

[1] Negligible output.
[2] Includes only iron excreted in sweat for 7.5 hours in environmental chamber. During the three overnight exposures at 100°F, the iron excreted in sweat averaged 0.102 mg/hour, a total of 1.68 mg for the 16.5-hour exposure.

TABLE 8

Phosphorus balance, with and without sweat losses; mean for three men for 16 consecutive days (1961 study)

Days at 100°F	Intake	Output			Balance	Sweat as % of total output
	Food	Urine	Feces	Sweat		
	mg/day	*mg/day*	*mg/day*	*mg/day*	*mg/day*	
1–4	1405	770	9.8	9.9	+625	1.2
5–8	1405	891	7.8	6.1	+500	0.7
9–12	1405	896	8.7	3.4	+497	0.4
13–16	1405	868	8.1	—	+529	—

TABLE 9

Total daily excretion of minerals; mean for three men (1961 study)

	Total excretion		7.5 hours	16.5 hours	Sweat excretion as % of total excretion	
	7.5 hours	16.5 hours[1]			7.5 hours	16.5 hours
	gm	*gm*	*mg/hour*	*mg/hour*		
Sodium	4.51	14.9	601	902	62.8	88.7
Potassium	0.94	1.39	125	84	30.1	52.0
	mg	*mg*				
Magnesium	17.0	29.7	2.3	1.8	12.4	27.5
Iron	0.98	1.68	0.131	0.102	4.5	11.0

[1] Minerals excreted in sweat during the regular 7.5-hour daily exposure and for three evenings when the men spent the remaining daily time (16.5 hours) at 100°F.

e sweat mineral losses. The sodium bal-ce varied due to the variation in daily ake (table 4). The potassium balance s positive when the sweat losses were cluded but were negative when the eat losses were included, averaging 1522, − 578, − 66 and − 195 mg/day the 4 consecutive 100°F periods (table . Magnesium balances did not vary eatly when comparing the inclusion and ion of the sweat losses, due to the ely high retention of magnesium 6). Iron balances, even though ey were reduced by approximately one-lf, were still on the low positive side able 7). The quantities of phosphorus sweat were so small in comparison to e total daily intake that they did not ange the highly positive balances sig-icantly (table 8).

The sweat losses during the 7.5-hour posure periods are presented as per-ntage of the total excretion in tables 4–Sodium in sweat accounted for 54 to %, potassium for 19 to 44%, magne-um for 10 to 15%, iron for 4 to 5% and osphorus for 0.4 to 1.2% of the total cretion of each respective element. If e included an average value for min-al losses in sweat during the remaining 5 hours of the day when the men were posed to 100°F temperature (days 7, and 15) the total daily mineral excre-on in sweat was approximately doubled able 9).

DISCUSSION

It has been mentioned previously that eat collections were made daily using e polyethylene bag, that covered one whole arm. The arm sweat which was representative for the entire period, was assumed to be comparable to the total body sweat. But a question arises whether this is a valid comparison. It has been shown by some investigators (Dill et al., '38; Johnston et al., '50; and Ladell, '48) that the various constituents of arm sweat are reasonably representative of the total body sweat. Van Heyninger and Weiner ('52) on the other hand are in disagreement, observing that the arm sweat is more concentrated than the rest of the body. Another factor to be considered is whether the arm bag will cause depression of the sweat rate. Collins and Weiner ('62) have observed a rapid depression of sweat gland activity in the forearm when the arm was covered with a sweat collection bag. Data are presented showing that the sweat rate is considerably less in humid environments than in dry conditions. These authors believe that the sweat depression in the arm bag may have been due to obstruction of the sweat gland ducts, by the excessive skin hydration.

Sodium is essential for the normal functioning of the body since it contributes to the acid-base balance of the body and since it is responsible in large measure, for the total osmotic pressure of the extracellular fluids (National Research Council, '58). Minimal allowances have not been established by the NRC due to lack of sufficient information, but it has been observed that a normal American intake will range from 3 to 7 gm of sodium/day. It has been reported (NRC, '58) that under normal conditions 90 to 95% of the

sodium intake is excreted in the urine and usually sweat sodium losses are not considered in sodium balance studies, under these conditions. Sodium can be excreted in sweat in fairly large quantities to disturb homeostasis and as a result seriously impair the economy of the organism (Schwartz, '60). Sodium excretion in sweat is variable, being dependent on physical activity, acclimatization, adrenal cortical activity, environmental temperature, humidity and body temperature. Under conditions of this study, where the daily sodium intake ranged from 8 to 10 gm/day, the losses of sodium in sweat accounted for more than 50% of the total daily excretion. Since the salt intake was ad libitum it was not possible to study the full effects of acclimatization on sodium excretion, but it was observed that the sweat sodium decreased appreciably during the first 12 days exposure to heat, which is in agreement with data reported in the literature. The sodium balance was highly positive by more than 3 gm even with the inclusion of the losses in sweat.

Potassium has been shown to be a required nutrient and is one of the principle basic elements in intracellular fluid, being found in greatest quantities within the cell. It is also a very important extracellular fluid constituent since it can influence muscular activity, notably the cardiac muscle, and can affect the excitability of the nerve tissue. Muscle protein repletions may demand an additional potassium intake of 2.7 mEq for each gram of nitrogen. The NRC ('58) has not established minimal allowances for potassium but a normal United States diet usually contains from 2.4 to 4.5 gm. An intake between 0.8 to 1.3 gm of potassium/day would be very close to the daily recommended allowances (NRC, '58). Potassium deficiencies, manifested by muscular weakness, increased nervous irritability, mental disorientation and cardiac irregularities, can be produced by gastrointestinal losses, by renal losses or by low potassium intake, and are frequently accompanied by metabolic alkalosis. The data in the literature are too limited to draw any specific conclusions on the relationship of potassium excretion in sweat and the total daily potassium excretion, as they may be related to such variables as body temperature, acclimatization to extreme heat, sweat rate and physical exercise. In some instances appreciable quantities of potassium in sweat have been reported (Schwartz, '60; Altman and Dittmer, '61) in man, living and exercising in extremely hot environments, but Davidson et al. ('59) and the British Ministry of Agriculture ('59) believe that sweat potassium losses are usually negligible, in relation to the daily intake. The potassium excreted in sweat accounted for approximately 30% of the total daily excretion. If the potassium losses in sweat for the remainder of the day were included, these losses could account for up to 50% of the total daily excretion. The potassium balances, with the addition of the sweat potassium losses, were all on the negative side.

Even though magnesium is a very important cellular constituent, there is very limited information in the literature in relation to magnesium metabolism in the body. It is known that for normal function, cardiac and skeletal muscles and nervous tissue depend greatly on a proper balance between calcium and magnesium ions. Magnesium will replace the calcium in bone salts when there is a calcium deficiency, but magnesium in excess will inhibit calcification. Even though the NRC has set up no minimal allowance, it has been calculated that the daily magnesium intake of adults in the United States is between 250 to 350 mg/day (NRC, '58).

Unlike sodium and potassium, the magnesium excretion in sweat did not decrease appreciably during acclimatization. The sweat accounted for approximately 12.5% of the total daily excretion of magnesium but when the overnight sweat losses are included, this excretion could account for 25% of the total. This sweat loss did not greatly affect the magnesium balance since the daily retention was approximately 200 mg/day. This retention may be in part compensation for the negative calcium balance reported in the same study (Consolazio et al., '62a), or it may be that the estimate of the minimal daily allowances of 250 to 300 mg of magnesium daily may be too high. Under the conditions of this study, the magnesium loss in sweat is relatively unimportant, but in studies of

magnesium intakes, these losses should considered.

ven though a few studies have been formed on the relationship of sweat es of iron to the daily requirements, quantitative importance of the cutane- iron losses is still a disputed issue. It he feeling of one group (Foy and Kondi, that the inclusion of the integumental losses may possibly increase the requirements by as much as 50% er non-stress conditions, and that these essive sweat losses in hot environments be a contributing factor in the devel- ent of iron deficient anemias. On the r hand Hussain and Patwardhan ('59) that the body tends to conserve iron he anemia state by reducing the losses ugh the skin. Dubach et al. ('55) us- radio iron as a tracer observed that ng maximum sweating in normal hu- ns, from 0.33 to 0.52 mg of iron may lost in sweat in a 24-hour period.

omparisons have been made on the rich and cell-free sweat iron (Hussain Patwardhan, '59) showing that the rich portion is from 3 to 10 times more centrated than the cell free sweat. It concluded that the major portion was ent in the products of cellular desqua- ion; and since active thermal sweating ways accompanied by cell desquama- the loss of iron in sweat could be of greater importance than is generally osed.

on loss via the sweat amounted to ap- imately 1 mg during the exposure od, which was 4.5% of the total daily etion. If the iron losses in sweat dur- the remainder of the day are included, could account for as much as 11% e total daily excretion.

ith a fairly high iron intake of 23.4 day, the iron balance was positive by oximately 1 mg/day when the sweat losses were included. These sweat excretions are fairly high, especially e includes the evening losses.

or to the beginning of the study the subjects were on leave at home for a -week interval. Hence, it is assumed they were eating a normal diet which have been comparable to the experi- tal diet. With the exception of potas- all of the mineral balances were positive. Under these conditions, the subjects could have been increasing body mass, but this may not be probable since the subjects lost a total of 1.05 kg during the experimental periods at 100°F (table 10).

TABLE 10

Body weight changes; mean change for three men for each 4-day period (1961 study)

	Days	Mean change in weight	
		kg/period	*kg/day*
Control period	1–4	+0.14	
	5–8	+0.49	
			+0.08
100°F period	1–4	+0.17	
	5–8	−0.21	
	9–12	−0.64	
	13–16	−0.37	
			−0.066
Recovery period	1–4	+0.77	
	5–8	−0.15	
			+0.08

It has been observed that sodium and potassium excretion in sweat decreased appreciably after acclimatization which is in agreement with data reported in the literature (Bass et al., '55; Dill et al., '38). On the other hand neither iron nor magnesium excretion in sweat decreased appreciably during acclimatization.

The data suggest that the excretion of sodium, potassium and iron in sweat are quite appreciable, especially under conditions that produce profuse sweating. These mineral losses must be considered in balance studies otherwise balance and daily minimal allowance data would be greatly misinterpreted. Studies in the literature where "equilibrium" was attained, under conditions of profuse sweating, should be reevaluated.

The concentration of phosphorus in sweat was found to be the lowest of any of the minerals analyzed in this study; averaging less than 0.5% of the total daily excretion. These values, which agree fairly well with data from other investigators, are relatively unimportant in computing balance studies.

The total osmolarity of sweat (freezing point) was decreased after acclimatization ranging from 116 to 141 milliosmoles after the acclimatization period. These values are comparable to other values for human sweat reported in the literature, and re-

viewed by Adams et al. ('58). These authors concluded that the osmotic concentration of sweat is hypotonic to serum and as the sweat increases, the osmotic concentration usually decreases.

SUMMARY

The results of this study show that a considerable quantity of sodium, potassium, magnesium and iron are lost in sweat of men during 16 consecutive days' exposure to environmental temperatures of 100°F. During a 7.5-hour collection period, the sweat excretions averaged 0.601 gm/hour for sodium, 0.125 gm/hour for potassium, 2.3 mg/hour for magnesium and 0.13 mg/hour for iron. Very small quantities of phosphorus are excreted in sweat, averaging between 0.45 to 0.81 mg/hour.

In the past, with the exception of sodium, very few investigators have recognized the fact that the mineral losses in sweat could be appreciable and as a result these losses have, all too frequently, been neglected in computing mineral balance. This could result in misinterpretation of the data, especially under conditions of profuse sweating. The total mineral loss should include the mineral loss in sweat. This in turn would help in estimating more realistically the minimal daily allowances of minerals.

ACKNOWLEDGMENTS

We wish to express our sincere appreciation and thanks to the following members of the Bioenergetics Division SSG J. B. Torres, SP5 O. Tarnowieckyi, SP5 G. L. Beal, SP4 E. E. Preston, SP4 S. D. Wilkins, SP5 L. J. Maland, SP4 W. E. Hendricks and SP4 L. E. Jones.

We are particularly indebted to the University of Colorado and Dr. Norman F. Witt, and are especially thankful for the full cooperation of the test subjects, without whom this study could never have been performed. They include Levi M. Yoder, Warren E. Kennel and Fred J. Eicker.

Our sincere thanks to Gerhard J. Isaac of the Statistics Branch, for setting up the experimental designs and schedules in these experiments.

LITERATURE CITED

Adams, R., R. E. Johnson and F. Sargent, II 1958 The osmotic pressure (freezing point) of human sweat in relation to its chemical composition. Quart. J. Exp. Physiol., 43: 241.

Adolph, E. F., and Associates 1947 Physiology of Man in the Desert. Interscience Publishers, Inc., New York.

Altman, P. M., and D. S. Dittmer 1961 Biological Handbooks. Blood and other body fluids. Federation of American Societies for Experimental Biology, Washington, 25, D. C.

Baird Associates 1953 Flame Photometer Manual, ed. 3., Cambridge, Massachusetts.

Bass, D. E., C. R. Kleeman, M. Quinn, A. Henschel and A. H. Hegnauer 1955 Mechanism of acclimatization to heat in man. Med., 34: 323.

Collins, K. J., and J. S. Weiner 1962 Observations on arm bag suppression of sweating and its relationship to thermal sweating "fatigue." J. Physiol., 161: 538.

Consolazio, C. F., and R. E. Johnson 1960 Biochemical and dietary procedures. U. S. Army Medical Research and Nutrition Laboratory Rep. no. 242. Denver, Colorado.

Consolazio, C. F., L. O. Matoush, R. A. Nelson, L. R. Hackler and E. E. Preston 1962a Calcium in sweat and its possible relation to calcium requirements. J. Nutrition, 78: 78.

Consolazio, C. F., R. A. Nelson, L. O. Matoush, R. S. Harding and J. E. Canham 1962b The nitrogen excretion in sweat and its relation to nitrogen balance and requirements. U. S. Army Medical Research and Nutrition Laboratory Rep. no. 270, Denver, Colorado.

Davidson, S., A. P. Meikeljohn and R. Passmore 1959 Human nutrition and dietetics. The Williams and Wilkins Company, Baltimore, Maryland.

Dill, D. B., F. G. Hall and H. T. Edwards 1938 Changes in composition of sweat during acclimatization to heat. Am. J. Physiol., 123: 412.

Dubach, R., C. V. Moore and S. Callender 1955 Studies in iron transportation and metabolism IX. The excretion of iron as measured by the isotope technique. J. Lab. Clin. Med., 45: 5[illegible]

Fiske Associates 1954 The Fiske Osmometer Instruction Manual, Boston, Mass.

Foy, H., and A. Kondi 1957 Anemias of the tropics in relation to iron intake, absorption and losses during growth, pregnancy and lactation. J. Trop. Med. Hyg., 60: 105.

Hussain, R., and V. N. Patwardhan 1959 Iron content of thermal sweat in iron-deficiency anaemia. Lancet, 276: 1073.

Johnston, F. A., T. J. McMillan and E. R. Evans 1950 Perspiration as a factor influencing the requirements for calcium and iron. J. Nutrition, 42: 285.

Ladell, W. S. S. 1948 The measurement of chloride losses in the sweat. J. Physiol., 107: 465.

Ministry of Agriculture, Fisheries and Food 19[illegible] Manual of Nutrition, ed. 5. Her Majesty's Stationery Office, London, England.

[illegible]bell, H. H., and M. Edman 1962 Nutri[illegible]nal significance of dermal losses of nutri[illegible]nts in man, particularly of nitrogen and min[illegible]rals. Am. J. Clin. Nutrition, *10:* 162.

[illegible]bell, H. H., and T. S. Hamilton 1949 The [illegible]ermal excretion under controlled environ[illegible]ental conditions of nitrogen and minerals [illegible] human subjects with particular reference to [illegible]alcium and iron. J. Biol. Chem., *178:* 345.

[illegible]onal Research Council, Food and Nutrition [illegible]oard 1958 Recommended Dietary Allowances, pub. 589, National Academy of Sciences — National Research Council, Washington, D. C.

Schwartz, I. L. 1960 Extrarenal regulation with special reference to the sweat glands. In Mineral Metabolism, an Advanced Treatise, eds., C. L. Comar, and F. Bronner. Academic Press, New York.

van Heyninger, R., and J. S. Weiner 1952 A comparison of arm-bag sweat and body sweat. J. Physiol., *116:* 395.

J. Water Pollution Control Fed., 37(9): 1308-1316, 1965

TOXICITY OF SELECTED CHEMICALS TO CERTAIN ANIMALS

Bobby F. Dowden and Harry J. Bennett

The median tolerance limit, defined as that concentration of a substance which is lethal to 50 percent of test animals in an arbitrary time period and abbreviated TL_m, has many uses in physiological studies. These values are especially useful as a starting point for studies of sublethal effects of chemicals on the metabolic processes of animals. Other uses may be made of these values depending on the nature of the research involved.

During the past 19 years the Industrial Research Laboratory at Louisiana State University has measured, for various purposes, the median tolerance limits of a large number of organic and inorganic chemicals for various animals. This paper presents the TL_m values of 86 of these chemicals as tested for 9 species of animals in 3 different support media. Some of the TL_m values were determined in conjunction with other research projects and, thus, were reported with that work (1) (2) (3). All the values reported herein have been gleaned from the Progress Reports of the Industrial Research Laboratory to the Louisiana Petroleum Refiners' Waste Control Council which have a limited circulation in the Council minutes to member organizations only. Because of the varied purposes for obtaining them, there can be little correlation of these data.

Bobby F. Dowden is Research Associate, Industrial Research Laboratory, and Harry J. Bennett is associated with the Department of Zoology, both at Louisiana State University, Baton Rouge.

Materials and Methods

The animals used for bioassay were amphipoda (probably *Hyallela* sp.); frog eggs, *Rana* sp.; three species of fish, *Carassius carassius, Lepomis marochirus,* and *Mollienesia latipinna;* mosquito larvae, *Culex* sp.; planaria worms, *Dugesia* sp.; snail eggs, *Lymnaea* sp.; and water fleas, *Daphnia magna.* The amphipods, planaria, and frogs were obtained from University Lake on the campus of the University. The snails from which eggs were obtained were found in a ditch near Fountainebleau State Park, 60 miles (96.6 km) east of Baton Rouge. The goldfish, *C. carassius,* and the mollies, *M. latipinna,* were obtained from a local pet shop. The bluegills, *L. macrochirus,* were obtained from state and federal fish hatcheries. The mosquito larvae were obtained during the summer in mixed culture of mostly *Culex pipiens* from puddles in a ditch on the campus. The daphnids were cultured in the laboratory from a starting culture originally obtained from Put-In-Bay, Ohio.

Bioassays with the fish were conducted by methods outlined by Freeman (4). Bioassays with the invertebrates and the frog eggs were conducted with the methods of Anderson *et al.* (5). These methods are basically similar to each other—they differ only in the dilution series utilized.

The support media employed were Standard Reference Water (4), Reference Dilution Water (6), and water from University Lake filtered through

glass wool. Standard Reference Water is described as a laboratory-prepared medium, free from organics, and containing all the major ions in concentrations and proportions of a mean surface water of the United States. Reference Dilution Water is a more easily formulated organic-free medium which will support aquatic animals longer than other artificial media tested.

Results and Discussion

The median tolerance limits determined are shown in Table I according to the chemicals tested. The animals on which the chemicals were tested, the support media employed, and the time for which the TL_m's were determined also are shown. In certain instances in the table, the same animal may be listed more than once under one chemical. This is because more than one support medium was employed. A different support medium may evoke a very different TL_m (7).

It may be noted in Table I that in the testing of combinations of chemicals, more than one concentration is given at one TL_m time period. These TL_m values are the test solution concentrations of each of the chemicals, separated by a hyphen, given in the order listed at the left side of the column.

Some values are given with the sign > (greater than) and < (less than). The TL_m values shown with either of these symbols preceding a single number, e.g., <3,750, represent either the upper or lower limit (whichever is applicable) of the most definitive test. A general rule used in the laboratory is that with TL_m values greater than 3,000 mg/l the toxicity is due to hypertonicity of the test solution. The values with two numbers and two symbols, e.g., >100 <1,000, designate the limits or "brackets" determined from preliminary tests. The TL_m of the chemical lies between the values given. The TL_m values are presented in mg/l. If, at room temperature, the chemical were a solid, the measure of TL_m was by weight. If the chemical tested were a liquid at room temperature, the measure was by volume.

It generally is expected that shorter-term TL_m values will be greater than those for longer periods of time. In Table I, it may be noted that some TL_m values for longer time periods are greater than those for shorter periods. This has occurred possibly because the TL_m values for one TL_m time period may have been tested by one worker at one time and the value for another time period tested by another worker at another time. Because the methods used by all the workers in the laboratory have been the same, these differences are considered to be due to differences in the test animals obtainable at the different times of testing.

Another consequence of having different workers determine the different TL_m's at different times is a lack of strict comparability of the data presented. In most cases, these data were obtained as background material for other research problems, e.g., effects of certain chemicals on the taste of fish flesh, effects on adults emerging from larvae exposed to various chemicals, effects on the young after sublethal exposure of the eggs to certain chemicals, and toxicity levels of common solvents used for transport or dilution of other chemicals. It is felt that the use of these data lies in their being utilized for the same purpose for which they were obtained, i.e., background material for more detailed studies.

The TL_m values given for the *Lymnaea* sp. eggs are the concentrations at which 50 percent of the eggs hatched. The *Lymnaea* snails used herein lay their eggs in an orderly arrangement of 8 to 12 eggs per egg case. This egg case is more or less rigid—having the consistency of a gela-

TABLE I.—Median Tolerance Limits of Certain Chemicals for Various Animals in Different Dilution Media

Compound and Animal Tested	Medium	TL_m (mg/l)			
		24 hr	48 hr	72 hr	96 hr
Acetic acid					
Culex sp. larvae	RDW	1,500	1,500	—	—
D. magna	SRW	47	—	—	—
L. macrochirus	RDW	>100<1,000	—	—	—
Acetone					
D. magna	RDW	10	10	—	—
Adipic acid					
L. macrochirus	RDW	<330	—	—	—
Ammonium chloride					
C. carassius	SRW	640	—	—	—
D. magna	ULW	202	161	67	50
D. magna	SRW	—	—	—	139*
L. macrochirus	SRW	725	725	725	725
Lymnaeae sp. egg	ULW	241	173	73	70
Ammonium hydroxide					
D. magna	SRW	60*	32*	—	20*
Ammonium sulfate					
D. magna	SRW	423*	433*	—	292*
Ammonium sulfite					
D. magna	SRW	299*	273*	—	203*
Barium chloride					
Rana sp. eggs	ULW	244.3 mg/l—91.67% development 2,443.0 mg/l—89.55% development 24,430.0 mg/l— 0.00% development			
Butyric acid					
D. magna	SRW	—	61	—	—
L. macrochirus	RDW	200	—	—	—
Calcium chloride					
D. magna	SRW	3,526*	3,005*	—	—
D. magna	ULW	1,838	759	759	649*
L. macrochirus	SRW	8,350	—	—	—
Lymnaea sp. eggs	ULW	4,485	3,094	3,308	2,573
Capric acid					
L. macrochirus	RDW	I			
Caproic acid					
L. macrochirus	RDW	>150<200	—	—	—
Caprylic acid					
L. macrochirus	RDW	I	—	—	—
Chromic sulfate					
D. magna	ULW	0.1	0.03	—	—
Chromic sulfate plus sodium dichromate					
Lymnaea sp. eggs	ULW	0.17—0.22	0.17—0.22	—	—

* Denotes 25 hr when placed after a value in the 24-hr column, 50 hr when in the 48-hr column, and 100 hr when in the 96-hr column.

Note:

TL_m (mg/l)—median tolerance limit.
SRW—Standard Reference Water (4).
RDW—Reference Dilution Water (3).
ULW—glass-wool filtered University Lake water.
I—chemical too insoluble in water to be toxic.

TABLE I.—*Continued*

Compound and Animal Tested	Medium	TL_m (mg/l)			
		24 hr	48 hr	72 hr	96 hr
Ferric chloride					
D. magna	SRW	36*	21*	—	15*
Formaldehyde					
D. magna	RDW	>100<1,000	—	—	—
Formic acid					
L. macrochirus	RDW	175	—	—	—
Glutaric acid					
L. macrochirus	RDW	330	—	—	—
Magnesium chloride					
D. magna	SRW	3,391*	3,699*	—	3,484*
Magnesium sulfate					
D. magna	ULW	963	929	861	788
D. magna	SRW	—	—	—	3,803
L. macrochirus	SRW	19,000	—	—	—
Lymnaea sp. eggs	ULW	10,530	6,525	6,300	6,250
Malonic acid					
L. macrochirus	RDW	150	—	—	—
Phenol					
D. magna	RDW	100	100	—	—
D. magna young	SRW	17*	7*	—	—
D. magna adult	SRW	61*	21*	—	—
L. macrochirus	SRW	>10<15*	—	—	—
M. latipinna	SRW	63*	22*	—	—
Potassium chloride					
D. magna	SRW	—	—	—	679*
D. magna	ULW	343	337	117	29
L. macrochirus	SRW	5,500	—	—	—
Lymnaea sp. eggs	ULW	1,941	1,492	1,018	1,100
Potassium cyanide					
D. magna	ULW	2	2	0.7	0.4
Lymnaea sp. eggs	ULW	796	796	147	130
Potassium dichromate					
C. carassius	SRW	705	—	—	—
D. magna	SRW	—	—	—	0.4*
L. macrochirus	SRW	739	—	—	—
Potassium ferricyanide					
D. magna	ULW	905	549	0.6	0.1
Potassium nitrate					
D. magna	ULW	490	490	226	39
D. magna	SRW	—	—	—	900*
L. macrochirus	SRW	5,500	—	—	—
Lymnaea sp. eggs	ULW	1,941	1,492	1,018	1,100
Propionic acid					
Culex sp. larvae	RDW	>1,000	>1,000	—	—
D. magna	SRW	—	50	—	—
L. macrochirus	RDW	188	—	—	—
Pyridine					
D. magna	RDW	2,114	944	—	—
Sodium acetate					
L. macrochirus	RDW	5,000	—	—	—
Culex sp. larvae	RDW	7,500	7,425	—	—
Sodium anthraquinone alpha-sulfonate					
D. magna	ULW	186	186	186	50
D. magna	SRW	—	—	—	12*
Lymnaea sp. eggs	ULW	186	186	186	186

TABLE I.—*Continued*

Compound and Animal Tested	Medium	TL_m (mg/l)			
		24 hr	48 hr	72 hr	96 hr
Sodium bicarbonate					
Culex sp. larvae	RDW	2,000	2,000	—	—
Sodium bisulfate					
Culex sp. larvae	RDW	300	300	—	—
Sodium bisulfite					
D. magna young	SRW	116*	81*	—	—
D. magna adult	SRW	—	—	—	102
D. magna adult	ULW	171	119	97	82
Dugesia sp.	ULW	179	179	179	179
Lymnaea sp. eggs	ULW	179	59	59	59
M. latipinna	SRW	241*	220*	—	—
Sodium bisulfite plus sodium carbonate					
D. magna	ULW	1,484–665	1,484–665	1,060–475	99–45
D. magna	SRW	—	—	—	436–85
Sodium bisulfite plus sodium chromate					
D. magna	SRW	—	—	—	67–0.278*
Sodium bisulfite plus sodium silicate					
D. magna	SRW	14,210–950	11,723–784	222–15	222–15
Sodium bisulfite plus sodium sulfate					
D. magna	SRW	—	—	—	82–3,654
Sodium bisulfite plus sodium carbonate and sodium chromate					
D. magna	SRW	—	—	—	86–441–0.354*
Sodium bisulfite plus sodium carbonate and sodium silicate					
D. magna	SRW	—	—	—	39–198–93*
Sodium bisulfite plus sodium carbonate and sodium sulfate					
D. magna	SRW	—	—	—	57–296–2,569*
Sodium bisulfite plus sodium chromate and sodium silicate					
D. magna	SRW	—	—	—	224–0.86–506*
Sodium bisulfite plus sodium chromate and sodium sulfate					
D. magna	SRW	—	—	—	78–0.32–3,443*
Sodium bisulfite plus sodium silicate and sodium sulfate					
D. magna	SRW	—	—	—	52–126–2,326*
Sodium p-bromobenzene sulfonate					
D. magna	ULW	2,347	1,943	971	809
D. magna	SRW	—	—	—	523*
L. macrochirus	SRW	<1,560	—	—	—
Lymnaea sp. eggs	ULW	2,590	2,590	2,590	2,590
Sodium butyl sulfonate					
D. magna	ULW	8,000	8,000	5,400	2,700

TABLE I.—*Continued*

Compound and Animal Tested	Medium	TL_m (mg/l)			
		24 hr	48 hr	72 hr	96 hr
Sodium butyrate					
L. macrochirus	RDW	5,000	—	—	—
Sodium carbonate					
Amphipoda	ULW	360	176	67	67
Culex sp. larvae	RDW	1,820	600	—	—
D. magna	ULW	347	265	—	—
D. magna	SRW	607*	565	—	524
Dugesia sp.	ULW	384	360	360	341
L. macrochirus	ULW	385	—	—	—
Lymnaea sp. eggs	ULW	403	403	395	411
M. latipinna	SRW	405*	297*	—	—
Sodium carbonate plus sodium chromate					
D. magna	SRW	—	—	—	420–0.34*
Sodium carbonate plus sodium chromate and sodium silicate					
D. magna	SRW	—	—	—	187–0.15–88
Sodium carbonate plus sodium chromate and sodium sulfate					
D. magna	SRW	—	—	—	240–0.19–2,078*
Sodium carbonate plus sodium silicate					
D. magna	ULW	265–1,776	265–1,776	265–1,776	265–1,776
Sodium carbonate plus sodium silicate and sodium sulfate					
D. magna	SRW	—	—	—	161–76–1,396*
Sodium carbonate plus sodium sulfate					
D. magna	ULW	198–666	172–577	172–577	66–222
Sodium chloride					
C. carassius	SRW	13,750	—	—	—
Culex sp. larvae	RDW	10,500	10,200	—	—
D. magna	SRW	6,447*	5,874*	—	3,114*
D. magna	ULW	3,412	3,310	—	—
L. macrochirus	SRW	14,125	—	—	—
Lymnaea sp. eggs	ULW	3,412	3,388	—	—
M. latipinna	SRW	18,735*	16,595	—	—
Sodium p-chlorobenzene sulfonate					
D. magna	ULW	8,600	7,659	3,964	2,150
D. magna	SRW	—	—	—	2,394*
L. macrochirus	SRW	<3,219	—	—	—
Lymnaea sp. eggs	ULW	8,600	7,633	6,343	5,053
Sodium 2-chlorotoluene-4-sulfonate					
L. macrochirus	SRW	<1,374	—	—	—
Sodium 2-chlorotoluene-5-sulfonate					
D. magna young	SRW	0.8*	0.6*	—	0.4*
D. magna adult	SRW	3.3*	1.3*	—	—
Lymnaea sp. eggs	SRW	30*	—	—	—
M. latipinna	SRW	115.2*	66.1*	—	—

TABLE I.—*Continued*

Compound and Animal Tested	Medium	TL_m (mg/l)			
		24 hr	48 hr	72 hr	96 hr
Sodium chromate plus sodium silicate					
D. magna	SRW	—	—	—	0.21–130*
Sodium chromate plus sodium silicate and sodium sulfate					
D. magna	SRW	—	—	—	0.28–122–2,255*
Sodium chromate plus sodium sulfate					
D. magna	SRW	—	—	—	0.28–3,044*
Sodium 2, 5-dichlorobenzene sulfonate					
D. magna	ULW	4,931	4,931	2,490	938
D. magna	SRW	—	—	—	1,468*
L. macrochirus	SRW	<3,750	—	—	—
Lymnaea sp. eggs	ULW	4,981	4,513	3,984	3,144
Sodium dichromate					
D. magna	ULW	22	10	—	—
Sodium formate					
L. macrochirus	RDW	5,000	—	—	—
Sodium nitrate					
C. carassius	SRW	12,150	—	—	—
D. magna	ULW	5,980	3,581	2,125	665
D. magna	SRW	—	—	—	4,206
L. macrochirus	SRW	12,800	—	—	—
Lymnaea sp. eggs	ULW	6,375	6,460	5,950	3,251
Sodium m-nitrobenzene sulfonate					
D. magna	ULW	8,665	8,665	6,017	5,067
D. magna	SRW	—	—	—	2,235*
L. macrochirus	SRW	<1,350	—	—	—
Sodium 4-nitrochlorobenzene-2-sulfonate					
D. magna	ULW	4,698	3,483	948	948
D. magna	SRW	—	—	—	1,474
L. macrochirus	SRW	6,375	—	—	6,375*
Lymnaea sp. eggs	ULW	3,532	4,439	3,736	3,208
Sodium 4-nitrotoluene-2-sulfonate					
L. macrochirus	SRW	<1,440	—	—	—
Sodium oxalate					
L. macrochirus	RDW (without $CaCl_2$)	4,000	—	—	—
Sodium p-phenolsulfonate					
D. magna	ULW	13,510	13,510	3,494	1,471
L. macrochirus	SRW	—	—	—	19,616*
Lymnaea sp. eggs	ULW	10,700	9,122	8,828	8,828
Sodium mono-hydrogen phosphate					
D. magna	SRW	1,154*	1,089*	—	426*
Sodium mono-hydrogen phosphate plus sodium pyrophosphate					
D. magna	ULW	3,580–433	3,580–391	—	—
Lymnaea sp. eggs	ULW	2,685–63	2,954–223	2,828–60	2,685–248

TABLE I.—*Continued*

Compound and Animal Tested	Medium	TL_m (mg/l)			
		24 hr	48 hr	72 hr	96 hr
Sodium phosphate					
D. magna	SRW	237*	177*	—	126*
Sodium propionate					
Culex sp. larvae	RDW	—	2,320	—	—
L. macrochirus	RDW	5,000	—	—	—
Sodium pyrophosphate					
D. magna	ULW	433	391	—	—
Sodium silicate					
Amphipoda	ULW	895	263	261	160
D. magna	ULW	575	494	413	216
D. magna	SRW	—	—	—	247
Lymnaea sp. eggs	ULW	632	630	630	632
Sodium sulfate					
Amphipoda	ULW	2,380	1,110	880	880
Culex sp. larvae	RDW	11,430	13,350	—	—
D. magna	ULW	8,384	2,564	725	630
D. magna adult	SRW	—	—	—	4,547
D. magna young	SRW	6,800	6,100	—	—
L. macrochirus	SRW	17,500	—	—	—
Lymnaea sp. eggs	ULW	5,401	5,400	5,400	3,553
M. latipinna	SRW	20,040	15,996	—	—
Sodium sulfide					
D. magna	SRW	16*	13*	—	9*
Sodium sulfite					
D. magna	SRW	299*	273*	—	203*
Sodium thiosulfate					
D. magna	SRW	2,245*	1,334*	—	805*
Sodium valerate					
L. macrochirus	RDW	5,000	—	—	—
Valeric acid					
D. magna	SRW	—	45	—	—
Xylene					
D. magna	RDW	>100 <1,000	—	—	—
Zinc stearate					
L. macrochirus	RDW	I			

tin dessert which has "set." It is felt that the more or less rigid case of *Lymnaea* may offer a small amount of protection from the chemicals tested, thereby giving the TL_m values almost equivalent to the values for adults of other forms.

One very significant effect of some of the chemicals tested herein has been largely unexplored. This is the effect of pH on the TL_m values. In one of the few definitive tests of pH effect conducted it was found that a 0.1 unit increase in pH increased the toxicity of ammonium chloride tenfold and that a 0.1 unit decrease reduced the toxicity by tenfold. The concentration of ammonium chloride was held at the TL_m concentration. This variation in toxicity probably is due to the logarithmic basis for pH. The pH and some other effects of some weak acids have been studied and reported to be due to the concentration of the undissociated portion of the molecule (7).

Summary and Conclusions

The median tolerance limits (TL_m) of 86 different chemicals and combinations of chemicals, both organic and inorganic, are given for 9 different animals in 3 different support media. Determination of the TL_m of a chemical at a different time may show vari-

ations from the expected because of differences in test animals available at the different times of testing. *Lymnaea* sp. eggs show TL_m values comparable to those of adults of other forms because of protection afforded by the egg case. Increasing or decreasing the pH of a concentration of a chemical can cause an increase or decrease in toxicity commensurate to the logarithmic basis for pH.

Acknowledgment

This research was conducted under a grant by the Louisiana Petroleum Refiners' Waste Control Council administered by the faculty of the Department of Zoology of Louisiana State University. Laboratory facilities were provided by the Division of Water Pollution Control of the Louisiana Wild Life and Fisheries Commission. Appreciation is expressed to each of the workers in the Industrial Research Laboratory who have aided in obtaining these data over the past 19 years.

References

1. Freeman, L., "Toxicity Thresholds of Certain Sodium Sulfonates for *Daphnia magna* Straus." *Sewage and Industrial Wastes*, 25, 11, 1331 (Nov. 1953).
2. Freeman, L., and Fowler, I., "Toxicity of Combinations of Certain Inorganic Compounds to *Daphnia magna* Straus." *Sewage and Industrial Wastes*, 25, 10, 1191 (Oct. 1953).
3. Dowden, B. F., "Cumulative Toxicities of Certain Inorganic Salts to *Daphnia magna* as Determined by Median Tolerance Limits." *Proc. La. Acad. Sci.*, 23, 77 (1960).
4. Freeman, L., "A Standardized Method for Determining Toxicity of Pure Compounds to Fish." *Sewage and Industrial Wastes*, 25, 7, 845 (July 1953).
5. Anderson, B. G., Andrews, T. F., Chandler, D. G., Jahoda, W. J., "The Evaluation of Aquatic Invertebrates as Assay Organisms for the Determination of the Toxicity of Industrial Wastes." Report of the Ohio State University Research Foundation to the American Petroleum Institute, New York (1948).
6. Dowden, B. F., "A Simple Solution for the Support of Aquatic Test Animals." *Proc. La. Acad. Sci.*, 25, 80 (1962).
7. Dowden, B. F., "Toxicity of Commercial Waste-Oil Emulsifiers to *Daphnia magna*." THIS JOURNAL, 34, 10, 1010 (Oct. 1962).
8. Betz, N. L., "Toxicity Tolerance Limits for Water Soluble Mono- and Dicarboxylic Acids to *Lepomis macrochirus* Rafinesque." Masters Thesis, Louisiana State University Library, Baton Rouge (1963).

MONTHLY CONTRACT AWARDS—WASTEWATER TREATMENT WORKS AND COLLECTING SEWERS AS REPORTED BY THE PUBLIC HEALTH SERVICE (MILLIONS OF DOLLARS)

Month	Treatment		Sewers		Total	
	Monthly	Cumulative	Monthly	Cumulative	Monthly	Cumulative
(1965)						
January	41.2	41.2	22.9	22.9	64.1	64.1
February	40.4	81.6	22.0	44.9	62.4	126.5
March	43.4	125.0	38.3	83.2	81.7	208.2
April	38.8	163.8	28.2	111.4	67.0	275.2
May	33.5	197.3	38.6	150.0	72.1	347.3
June	64.8	262.1	56.6	206.6	121.4	468.7
July	41.6	303.7	37.0	243.6	78.6	547.3

THE EFFECTS OF ORAL ADMINISTRATION OF MAGNESIUM OXIDE ON RENAL CALCIUM EXCRETION IN CREW MEMBERS DURING A SUBMARINE PATROL

by

LCDR Clayton T. Drake, MC, USN

SUBMARINE MEDICAL RESEARCH LABORATORY
NAVAL SUBMARINE MEDICAL CENTER REPORT NO. 601

Bureau of Medicine and Surgery, Navy Department
Research Work Unit MF099.01.01.04

Submitted by

John H. Baker, CDR MC USN
Director, School of Submarine Medicine

Reviewed and Approved by:

Charles F. Gell, M.D., D.Sc.(Med)
Scientific Director
SubMedResLab

Reviewed and Approved by:

J. D. Bloom, CDR MC USN
Director
SubMedResLab

Approved and Released by:

J. E. Stark, CAPT MC USN
COMMANDING OFFICER
Naval Submarine Medical Center

This document has been approved for public release and sale; its distribution is unlimited.

1969 (rec'd 1970)

ABSTRACT

Twenty-four urine specimens were collected from randomly selected members of the crew of the U.S. Navy Submarine NATHAN HALE, SSBN-623, while at sea on a routine patrol mission. Specimens were collected three days after submergence, and again a few days before return to port. Twenty-one men received daily dosages of 310 mg of magnesium oxide orally, while nineteen served as controls. Urine specimens were examined microscopically, then measured for calcium, phosphorus, and routine factors.

No urine specimen examined was found to contain bacteria or white blood cells, nor was there any alteration in the routine chemistries.

Total urinary calcium excretion in the control group fell from a mean of 178 mg pre-patrol to 135 mg post-patrol, while the group ingesting magnesium oxide exhibited a sample mean of 162 mg calcium post-patrol, which is a significantly different mean value ($p=0.05$). Phosphorus excretion was not significantly variant.

These data indicate that magnesium oxide does have an ionic effect on urinary chemical excretion and may explain the so-called "protective mechanism."

TABLE OF CONTENTS

	Page
Introduction	1
Procedure	1
Results	2
Discussion	3
Summary	3
Conclusions	4
Acknowledgments	4
References	4
Addendum	4

THE EFFECTS OF ORAL ADMINISTRATION OF MAGNESIUM OXIDE ON RENAL CALCIUM EXCRETION IN CREW MEMBERS DURING A SUBMARINE PATROL

INTRODUCTION

The magnesium ion has long been suspected (1-5) of exerting a protective effect on calcium urolithiasis. An interrelationship between the two has not been clearly elucidated, but it is postulated that the protective mechanism has to do with a decrease in urinary calcium excretion (4). However, there is no clear-cut data to indicate that a high urinary calcium, per se, increases the risk of renal stone formation (6-9).

The controlled environmental conditions encountered aboard one of the Polaris submarines where subjects live in the same atmosphere, eat identical food, and, generally, have similar schedules of work and sleep, afforded an excellent opportunity to study the urinary excretion of calcium.

The submarine patrol by necessity causes many changes in daily lives: no sunlight, an almost completely sedentary existence, exposure to an atmosphere with an increased carbon dioxide content, and probable decreased water intake. These alterations might predispose the crew to a higher incidence of urinary tract stone formation.

Though it has been shown that respiration and acid-base balance are altered and that calcium mobilization does take place (10), no comprehensive data on calcium excretion after prolonged exposure to the submarine environment were available; therefore, this study was undertaken to determine whether significant increases in calcium and phosphorus excretion occurred and the "protective effect" of magnesium oxide in calcium oxalate stone formation.

PROCEDURE

For this study, forty healthy young men were followed for a period of approximately four months, in order to learn the effects of oral magnesium oxide on urinary calcium and phosphorus excretion. The project began with sixty volunteers, but those whose medical history or physical examination indicated renal abnormality or infection, or who were suspected of having had a renal stone were excluded. The forty men included in the study were all members of the U.S. Navy, between the ages of 19 and 33. Blood plasma specimens were screened for calcium, phosphorus, and uric acid levels, all of which were within normal ranges.

For a portion of a routine Polaris submarine patrol, these forty volunteers were observed. Several days after submergence, 24-hour samples of urine were collected and measured, and routine microscopic examinations were performed. Ten ml aliquots of urine preserved in dilute hydrochloric acid were frozen to be analyzed upon return to a laboratory facility. A second 24-hour urinary specimen was collected shortly before surfacing at the end of patrol. It was treated in the same manner. During the intervening period, twenty-one men ingested 310 mg of magnesium oxide each day, while nineteen served as controls. These men were closely matched in age, race, body build, type of job, amount of exercise, type and amount of food consumed, water intake, and all environmental conditions. None of the men studied suffered any illnesses or abnormal stresses during the period of study. All were examined physically at the completion of the patrol and found to be in excellent physical condition.

RESULTS

Routine urinalysis indicated that both groups, after patrol, tended to have a more dilute urine. This finding is not explained simply, but change in the acid-base balance is not suspect, as the urinary pH remained approximately constant.

Microscopic analysis was carried out on the post-patrol urines only (see Figure 1). Of interest here is that no leucocytes or bacterial forms were noted in any urine specimen. The percentage of urines with amorphous forms, epithelial cells, and crystals,

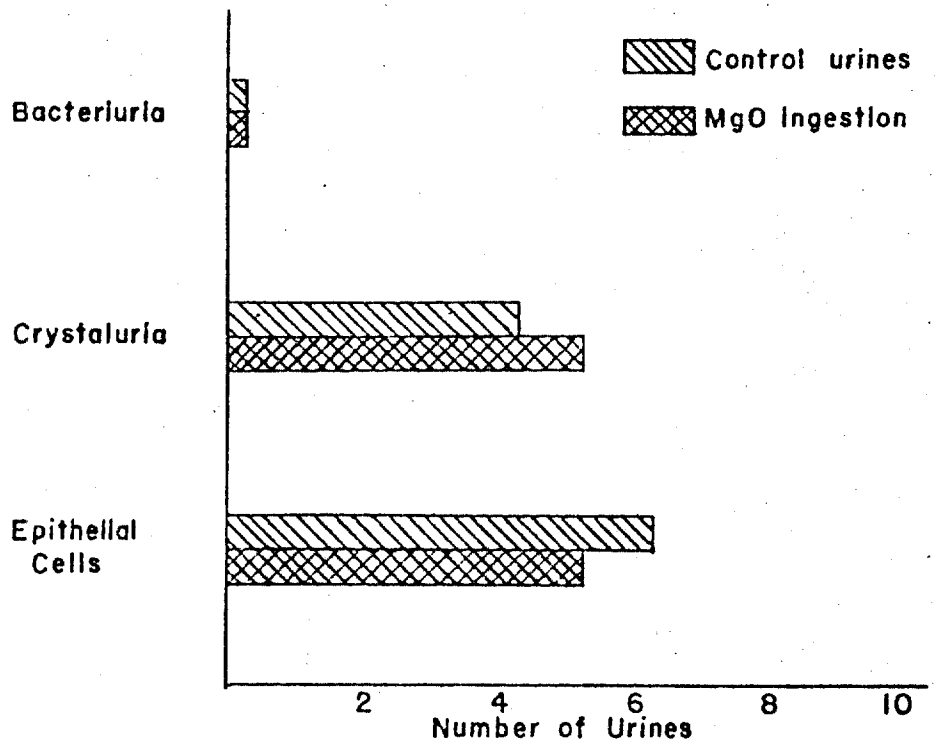


Figure I.— Microscopic Analysis

does not differ from an average Stateside population in routine analysis. Each specimen was tested for glucose and protein, both pre- and post-patrol. Neither was detected at any time.

Urinary volumes for the two groups were quite similar. The differences between pre- and post-patrol volumes were slight (Table I).

TABLE I.—Urinary Volume (24-hour samples) for MgO-Treated and Control Group

	Urinary Volume (cc) Mean	Stan. Deviation
MgO-Treated Group (N — 21)		
Pre-Patrol	1300	509
Post-Patrol	1338	657
Control Group (N — 19)		
Pre-Patrol	1226	419
Post-Patrol	1218	547

Data on calcium and phosphorus excretion are summarized in Tables II and III, respectively. For the MgO-treated group there was practically no difference in mean calcium excretion between the two sample periods, 168 mg pre-patrol compared with 167 mg post-patrol.

TABLE II.—Calcium Excretion (24-hour samples) for MgO-Treated and Control Group

	Calcium Excretion (mg) Mean	Stan. Deviation
MgO-Treated Group (N—21)		
Pre-Patrol	169.1	49.3
Post-Patrol	166.6	89.2
Amount of change, pre- to post-Patrol	2.5	70.0*
Control Group (N—19)		
Pre-Patrol	187.2	83.5
Post-Patrol	135.5	69.6
Amount of change, Pre- to Post-Patrol	51.6	88.3*

* Intercorrelation between repeated measurements taken into account.

In contrast, the mean excretion of calcium by the control group dropped from 187 mg to 136 mg post-patrol, a decrease significant

at the ($p<.01$) level, by a t-test for repeated measurements. The difference between pre- and post-patrol change in calcium excretion by the two groups was significant at the ($p<.05$) level by a one-tailed t-test for repeated measurements ($t=1.88$).

Both groups showed slight drops in phosphorus excretion from the pre- to post-patrol samples (Table III), the drop being slightly more for the control group. The changes, however, were not statistically significant, nor was there a significant difference in changes between the two groups.

TABLE III.—Phosphorous Excretion (24-hour samples) for MgO-Treated and Control Group

	Phosphorous Excretion (mg) Mean	Stan. Deviation
MgO-Treated Group (N—21)		
Pre-Patrol	1018.6	345.7
Post-Patrol	974.6	478.0
Pre- to Post-Patrol Change	44.0	546.1*
Control Group (N—19)		
Pre-Patrol	967.2	420.2
Post-Patrol	912.1	374.4
Pre- to Post-Patrol Change	55.1	531.3*

* Intercorrelations between repeated measurements taken into account.

DISCUSSION

In the United States the overall incidence of urologic stone is put at 0.7 percent, or 7 cases per 1,000 population per year (11). One would expect, therefore, several cases of renal stone to occur among submariners each year. Any method of decreasing this incidence should be thoroughly evaluated.

Oxalate stones account for over 90 percent of the urolithiasis encountered in North America (12). In contrast to uric acid and cystine stones, which are formed in urines with high concentrations of these components, the mechanism of formation of the oxalate stone is not yet known. Many authors (13-16) have compared the urinary output of calcium, phosphorus, and oxalic acid in patients with stones to that of normal controls, finding no significant differences. However, other authors, noting the protective effects of magnesium, demonstrated that magnesium increases the solubility of calcium oxalate (17, 18). Takasaki and Shimano also measured urinary calcium, phosphorus, magnesium and oxalate. They concluded in an exhaustive study that the magnesium: oxalate ratio is the most important factor in oxalate stone formation. Any magnesium: oxalate ratio less than 2.0, or a magnesium: phosphorus ratio less than 0.1 (urinary oxalate and phosphorus were shown to be positively correlated) was said to be a "magnesium-oxalic acid unbalanced urine," in which stone formation would more likely take place (19).

The present study demonstrates that there is little change in urinary pH, sugar, protein, or sedimentation (Figures 1-4). Bacteriuria was not found in any urine tested. The volume of urine excreted by the submariners was found to average quite a bit less than the average normal adult excretion rate of 2-3 liters/24 hours. This finding reflects the stasis of the individual and the stability of his environmental conditions, and is a factor which could lead to a higher incidence of renal stone formation.

Of greater interest are the total calcium and phosphorus excretion rates. Significant differences (p is less than 0.05) are noted between the pre- and post-patrol values of calcium. Also, the administration of magnesium oxide significantly alters the observed excretion values of calcium. It is postulated that through this mechanism of chemical excretion rate alteration, magnesium oxide exerts its protective influence on stone formation. It is suggested that spacecraft, with a much more limited environment than submarines, might possibly utilize these data in the preservation of health and the utilization of manpower.

SUMMARY

After encountering two cases of renal urolithiasis while serving aboard the USS NATHAN HALE, SSBN-623(G), the medical officer became interested in studying urinary mineral excretion in submarine personnel in-

volved in prolonged submergence. He realized that documented research in this area would be necessary to round out the existing body of knowledge of the effects of the submarine environment on submarine crews, and therefore planned and carried out the study reported herein.

In order to learn the effects of orally administered magnesium oxide on excretion of urinary calcium and phosphorus, a study was undertaken on a group of forty submariners while on a routine patrol. Approximately half of the group (21) received daily dosages of magnesium oxide, while the remainder of the group (19) served as controls.

This report reflects procedures and findings of that study. Significant alterations are reported, which, suggest that if further research confirms the author's hypothesis, and if the side effects of magnesium oxide are minimal, the drug may have some value on the medical supply table in the future for prevention of urinary stone.

CONCLUSIONS

In this study, exposure to the submarine environment causes no change in the results of the routine urinalysis. No Glaucosuria or proteinuria were noted, no pus cells or bacterial forms were found, and no unusual or large amounts of crystalline forms were noted.

In this study a decreased urinary calcium excretion was found in the control subjects. Magnesium oxide in this controlled pilot study led to an increased excretion of calcium in the urine. This finding does not agree with the "decreased urinary excretion" theory for the protective influence, but lends some support to the "magnesium-oxalate balance" theory.

ACKNOWLEDGEMENTS

Appreciation is extended to the Special Chemistry Laboratory Naval Medical School, National Naval Medical Center, Bethesda, Maryland, for providing facilities and assistance in urinary chemistry analyses. Particular appreciation to HMC R. Rossman, USN, HM2 J. Grinnell, USN, and HM2 R. Collins, USN, for their valuable assistance in processing the test samples.

Also thanks to Ralph A. Lockhart, SS, USN, USN NATHAN HALE, SSBN-623 (G), who rendered valuable assistance in this study.

Appreciation is expressed for the help received from the Personnel Selection Branch, Submarine Medical Research Laboratory, Naval Submarine Medical Center, in the review of data analysis and for editorial and publication assistance.

ADDENDUM

Since the time of the first draft of this paper, more studies have been published which pertain to oxalate renolithiasis. Resnick, et al., reports that an important factor in the stone formation is familial, through a polygenic mode of inheritance. In Volume 280 of **The New England Journal of Medicine**, Lemann, et al., discuss their study of urinary calcium excretion when a carbohydrate load is given. In a study structured much the same as that reported here, those with a family history of renal stone responded to oral sugar administration with a significantly higher calcium excretion.

These findings suggest that several factors influence the formation of renal oxalate stones, factors which, when discovered, can lead to better prophylaxis for our military forces.

REFERENCES

1. Moore, C. A., and Bunce, G. E.: Reduction in frequency of renal calculus formation by oral magnesium administration. **Invest. Urol.**, 2:7, 1964.
2. Hammersten, G.: On calcium oxalate and its solubility in the presence of inorganic salts with special reference to the occurrence of oxaluria. **C. R. Trav. Lab., Carlsberg**, 17:1, 1929.
3. Gershoff, S. N., Faragalla, F. F., Nelson, D. A., and Andrus, S. B.: Vitamin B_6 deficiency and oxalate nephrocalcinosis in the cat. **Amer. J. Med.**, 27:72, 1959.
4. Andrus, S. B., Gershoff, S. N., Faragalla, F. F., and Prien, E. L.: Production of calcium oxalate renal calculi in vitamin B_6—deficient rats. **Lab. Invest.**, 9:7, 1960.

5. Lyon, E. S., Borden, T. A., Ellis, J. E., and Vermeulen, C. W.: Calcium oxalate lithiasis produced by pyridoxine deficiency and inhibition with high magnesium diets. **Invest. Urol.**, 4:133, 1966.

6. Flocks, R. H.: Calcium and phosphorus excretion in the urine of patients with renal or ureteral calculi. **J. A. M. A.**, 113:1466, 1939.

7. Henneman, P. H.: Effects of high calcium intakes on renal function. **Fed. Proc.**, 18:1093, 1959.

8. Malm, O. J.: Adaptation to alterations in calcium intake. In **The Transfer of Calcium and Strontium across Biological Membranes**, p. 145. R. H. Wasserman, Editor. Academic Press, Inc., New York, 1963.

9. Modlin, M.: Urinary calcium in normal adults and in patients with renal stone: An interracial study. **Invest. Urol.** 5:49, 1967.

10. Shaefer, K. E., et al.: Environmental physiology of submarines and spacecraft. **Arch. Environ. Health**, 9:320, 1964.

11. Beesow, P. B., and McDermott, W.: **Textbook of Medicine**, 11th ed. W. B. Saunders Co., Philadelphia, 1963.

12. Vermeulen, C. W., Miller, G. H., and Sawyer, J. B.: Some non-surgical aspects of urolithiasis. **Med. Clinics N. Am.**, 39:281, 1955.

13. McIntosh, J. F., and Read, M. K.: Oxalic acid excretion in oxalate lithiasis. **J. Urol.**, 80:272, 1958.

14. Hodgkinson, A.: The urinary excretion of oxalic acid in nephrolithiasis. **Proc. Roy. Soc. Med.**, 51:970, 1958.

15. Dempsey, E. F., Forbes, A. P., Melick, R. A., and Henneman, P. H.: Urinary oxalate excretion. **Metabolism**, 9:52, 1960.

16. Mayer, G. G., Markow, D., and Karp, F.: Enzymatic oxalate determination in urine. **Clin. Chem.**, 9:334, 1963.

17. Miller, G. H., Vermeulen, C. W., and Moore, J. D.: Calcium oxalate solubility in urine: experimental urolithiasis. XIV. **J. Urol.**, 79:607, 1958.

18. Elliot, J. S., and Eusebio, E.: Calcium oxlate solubility: the effect of inorganic salts, urea, creatinine, and organic acid. **Invest. Urol.**, 3: 72, 1965.

19. Takasaki, E., and Shimano, E.: The urinary excretion of oxalic acid and magneisum in oxalate urolithiasis. **Invest. Urol.**, 5:303, 1967.

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
U.S. Naval Submarine Medical Center, Submarine Medical Research Laboratory

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP
N/A

3. REPORT TITLE
THE EFFECTS OF ORAL ADMINISTRATION OF MAGNESIUM OXIDE ON RENAL CALCIUM EXCRETION IN CREW MEMBERS DURING A SUBMARINE PATROL

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
Interim report on Research Work Unit

5. AUTHOR(S) (First name, middle initial, last name)
Clayton T. DRAKE, LCDR, MC, USN

6. REPORT DATE
28 October 1969

7a. TOTAL NO. OF PAGES
5

7b. NO. OF REFS
19

8a. CONTRACT OR GRANT NO.

b. PROJECT NO.
MF099.01.01

c.

d.

9a. ORIGINATOR'S REPORT NUMBER(S)
SubMedResLab Report No. 601

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

10. DISTRIBUTION STATEMENT
This document has been approved for public release and sale; its distribution is unlimited.

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY
U.S. Naval Submarine Medical Center
Box 600, Naval Submarine Base
Groton, Conn. 06340

13. ABSTRACT

In order to learn the effects of orally administered magnesium oxide on the excretion of urinary calcium and phosphorus, a study was undertaken on a group of forty submariners while on a routine patrol. Half of the group received daily dosages of magnesium oxide, while the other half served as controls. Twenty-four-hour specimens were collected from these subjects three days after submergence, and again a few days before return to port.

Urine specimens were examined microscopically, then measured for calcium, phosphorous and routine factors. No specimen examined was found to contain bacteria, or white blood cells, nor was there any alteration in the routine chemistries.

Total urinary calcium excretion in the control group fell from a mean of 178 mg in pre-patrol tests to 135 mg post-patrol, while the group ingesting magnesium oxide exhibited a sample mean of 162 mg in the post-patrol tests. These figures show a significantly different mean value (p - 0.05) These results, if substantiated by further studies, would argue strongly for the prophylactic use of magnesium for protection against the formation of urinary calculi.

DD FORM 1 NOV 65 1473 (PAGE 1)

S/N 0101-807-6801

UNCLASSIFIED
Security Classification

3ND PPSO 13152

Security Classification

14 KEY WORDS	LINK A ROLE	LINK A WT	LINK B ROLE	LINK B WT	LINK C ROLE	LINK C WT
Urinary mineral excretion in submarine personnel						
Magnesium oxide effects on renal calcium excretion						
Renal urolithiasis, magnesium for control of						

DD FORM 1 NOV 65 1473 (BACK)
(PAGE 2)

Security Classification

vasodilator action. More recently, Overbeck, Daugherty and Haddy (1969) have reported that infusions of magnesium sulphate into the brachial artery in man caused an increase in forearm blood flow.

Despite ample documentation that magnesium sulphate has a vasodilator action, the mechanism of this effect seems obscure. Mayes (1950) suggests that the drug paralyses nerve-muscle connections in the walls of arterioles and thereby relaxes these vessels and reduced blood pressure. This influence as to a possible action of magnesium sulphate on vasoconstrictor nerves led to the present study, and, in an attempt to clarify its vasodilator action, the drug was given by close arterial infusion and its effects on the blood vessels of the forearm and hand recorded, in the presence and absence of a number of vasoactive blocking agents.

Materials and Methods.

The subjects were normal volunteer medical students and one patient (W.J.A.) who had undergone a unilateral cervical sympathectomy 4 months previously for Raynaud's phenomenon in the hand. The forearm vessels of this patient showed a minimal response to the sympathetic stimulus of mental arithmetic (Blair, Glover, Greenfield and Roddie, 1959), and only a small constriction of these vessels was seen when a large dose of the indirectly acting sympathomimetic agent tyramine was infused intra-arterially into the part. It has been shown (Frewin and Whelan, 1968) that the constrictor action of tyramine in man is dependent on the presence of sympathetic nerves, and the small constrictor response of the patient's forearm blood vessels to the drug indicated that only a few vasomotor fibres remained. The results of physiological tests indicated that the hand vessels were completely sympathectomised.

The experiments were carried out at laboratory temperatures ranging from 21 to 28°, the subjects lying recumbent on a couch at least 30 min. before observations were made, during which time the recording apparatus was applied and the infusion needle inserted.

Forearm or hand blood flow was measured by venous occlusion plethysmography, using water-filled plethysmographs (Greenfield, 1954) maintained at a temperature of 34-35° for the forearm and 32-33° for the hand. Three or four records of flow were obtained each minute.

Intra-arterial infusions were given into the brachial artery at the elbow of one side through a 22-gauge needle inserted centrifugally into the artery. This was connected by a length of polyethylene tubing to a mechanically-driven syringe which delivered 2 ml. of saline per min. Saline (0·9% w/v sodium chloride) was infused during the control periods and also used as a vehicle for the drugs. The doses of drugs administered were such that systemic effects were not produced, making it possible to use the opposite uninfused limb as a control.

Per cent changes in forearm flow produced by drug infusions were calculated from the averaged flow values during the 2 min. before the infusion and the last 2 min. of the infusion period, by which time the responses to the drugs had become stable. Allowance was made for spontaneous variations in flow unrelated to drug action by assuming that, in the absence of the drug infusion, the infused and the control sides would have maintained the same relationship to each other as in the pre-infusion period (Duff, 1952). This correction was not used in the experiment where sympathetic nervous transmission to the infused side had been interrupted.

Superficial (skin) and deep (muscle) oxygen saturations were determined using the method described by Roddie, Shepherd and Whelan (1956, 1957b). Polyethylene catheters (Intracath, No. 17, Bardic) were inserted centrifugally into a deep and superficial vein in the cubital fossa and, following the inflation of a wrist cuff to 200 mm. Hg to exclude hand flow, sampling of the venous blood from skin and muscle commenced. The oxygen saturation of each sample was rapidly determined by estimating the percentage of light transmission

through each sample with a Unicam SP 1400 prism absorptiometer using a wavelength of 660 mμ and a fixed cuvette. The assumption was made that during the test procedure the metabolic activity, and therefore the oxygen requirement of the tissue, remained constant, and the changes in the oxygen saturation of the returning venous blood reflected changes in blood flow through the tissue. A rise in the oxygen saturation therefore indicated an increase in blood flow and a decrease in oxygen saturation a fall in flow in the vessels concerned.

The drugs used were magnesium sulphate ($MgSO_4 . 7H_2O$), calcium gluconate (Farmer Hill Pty. Ltd.), histamine acid phosphate (David G. Bull Laboratory Pty. Ltd.), mepyramine maleate (Anthisan, M. & B.), propranolol hydrochloride (Inderal, I.C.I.), isoprenaline hydrochloride (Isuprel, Winthrop), acetylcholine chloride (Acetylcholin, Roche) and hyoscine hydrobromide (David G. Bull Laboratory Pty. Ltd.). The doses of drugs are expressed as weights of their salts.

RESULTS.

The dilator action of magnesium sulphate on the vessels of the forearm of one subject is illustrated in Fig. 1. Doses of 20 and 50 mg./min. infused into the brachial artery of one side for 10 min. caused a rise in blood flow which was greater with the larger dose. There was no effect on the vessels of the opposite forearm. The effect of magnesium sulphate on the hand blood vessels was to produce a dilatation of this vascular bed though the dose of the salt required was much greater than that needed to produce dilatation in the forearm. These large doses of magnesium sulphate which had to be infused to produce an increase in flow in the hand invariably produced dilatation of the hand vessels on the opposite side, indicating that the drug was exerting a systemic effect. The subsequent studies were therefore confined to the forearm blood vessels.

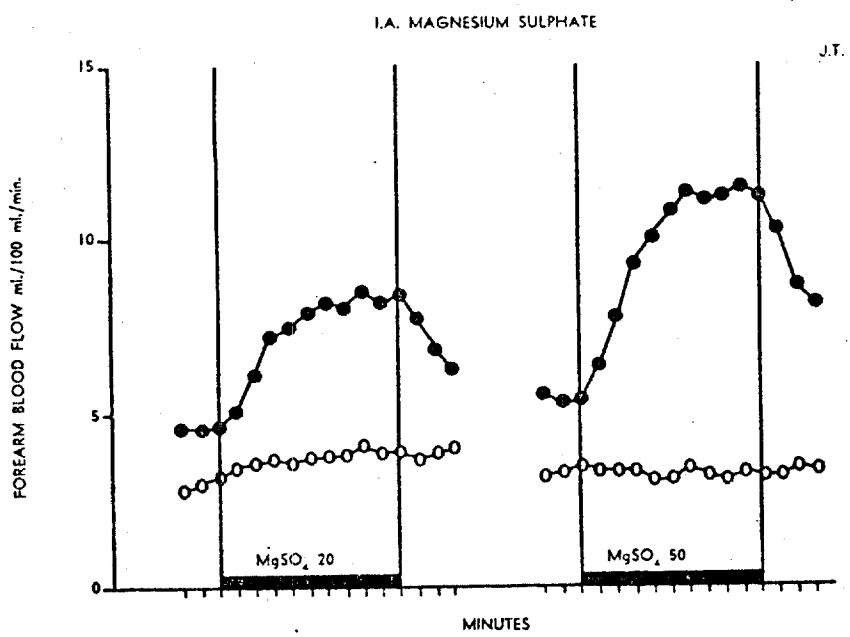


Fig. 1. The effect on blood flow through the forearm of two doses of magnesium sulphate infused into the brachial artery in a normal subject. ●, infused side; ○, control side. The 10 min. periods of drug infusion are indicated by the black rectangles, above which are shown the doses of magnesium sulphate in mg./min.

The data from eight experiments with magnesium sulphate on 8 different subjects are illustrated in Fig. 2. Five of these showed a strikingly greater increase in forearm flow when the dose of 50 mg./min. was infused intra-arterially, while in the other 3 the dilatation produced by the large dose was not much greater than that produced by the dose of 20 mg./min.

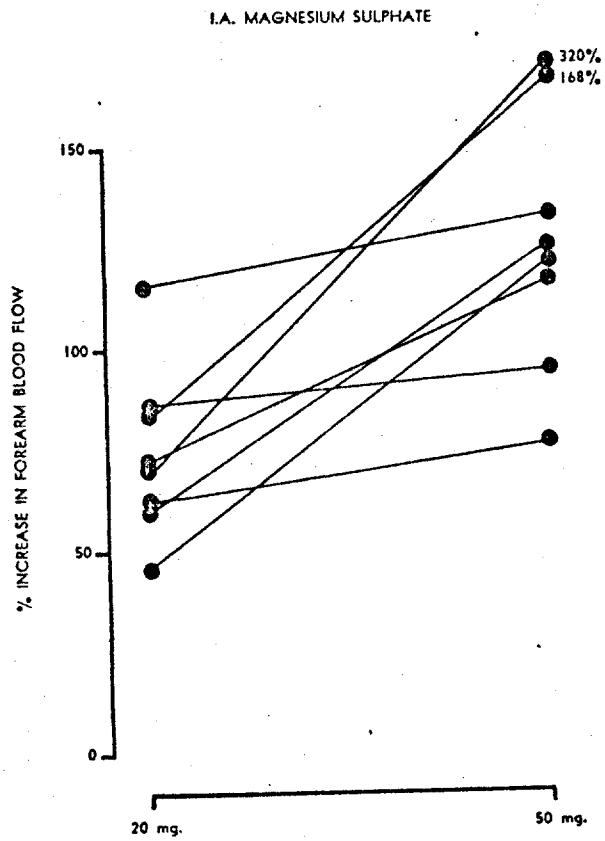


Fig. 2. Forearm blood flow during the last 2 min. of an intra-arterial infusion of magnesium sulphate expressed as the percentage increase from the pre-infusion level. The values to the left of the Fig. are the percentage increases in flow caused by an infusion of 20 mg./min., and those to the right of the Fig. the percentage increases in flow produced by infusion of 50 mg./min. of magnesium sulphate.

The effect of the β-receptor antagonist propranolol on the forearm vascular response to magnesium sulphate was studied in two experiments, the results of one of which are shown in Fig. 3. The left hand frame shows that the vasodilator effect of magnesium sulphate was not blocked by propranolol. The frame on the right shows that the same dose of propranolol blocked the vasodilator effect of isoprenaline. A similar result was obtained in the other subject. In both these experiments the second infusion of magnesium sulphate was administred 14 min. after the infusion of propranolol was completed.

The left hand frame of Fig. 4 portrays the fact that hyoscine did not affect magnesium sulphate's dilator action on the forearm vessels. To the right of the

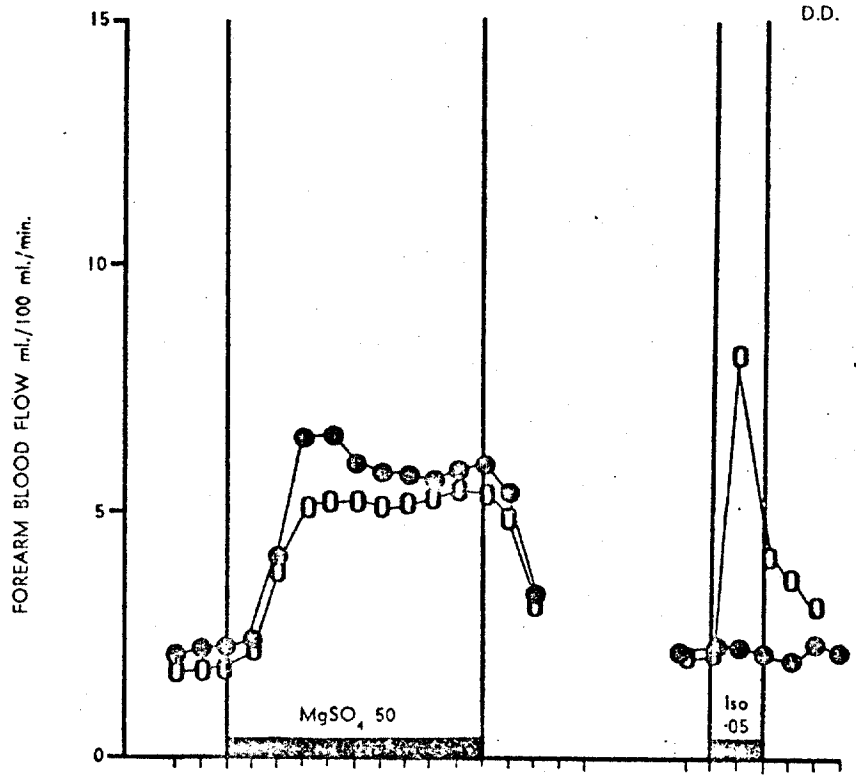


Fig. 3. The effect of propranolol intra-arterially (100 μg./min. for 8 min.) on the responses of the forearm blood flow of 1 subject to intra-arterial magnesium sulphate, the dose of which in mg./min. is indicated by the number above the black rectangle. ●, treated forearm; ○, untreated forearm. The frame to the right of the Fig. is the response of the forearm vessels to isoprenaline (·05 μg./min.) ○, before; ●, after propranolol.

Figure is shown the abolition of acetylcholine's dilator action by the same dose of hyoscine (0·1 mg./min.) administered intra-arterially for 4 min. This experiment was performed on another subject and a similar result obtained. In one of the experiments the second infusion of magnesium sulphate was administered 13 min. after the infusion of hyoscine was completed, and in the other it was given 6 min. after the hyoscine.

In two further experiments of similar design it was found that the antihistaminic drug mepyramine did not diminish magnesium sulphate's dilator action on the forearm blood vessels.

Fig. 5 shows the results of one of two experiments performed to test the response of the forearm blood vessels to a mixture of magnesium sulphate and calcium gluconate infused together into the brachial artery in equimolar doses. The first frame illustrates the dilator response to an infusion of 20 mg. of magnesium sulphate per min., while the middle frame shows the constriction produced by an infusion of 40 mg. of calcium gluconate per min. The third frame demonstrates the response of the forearm blood flow to infusing the calcium

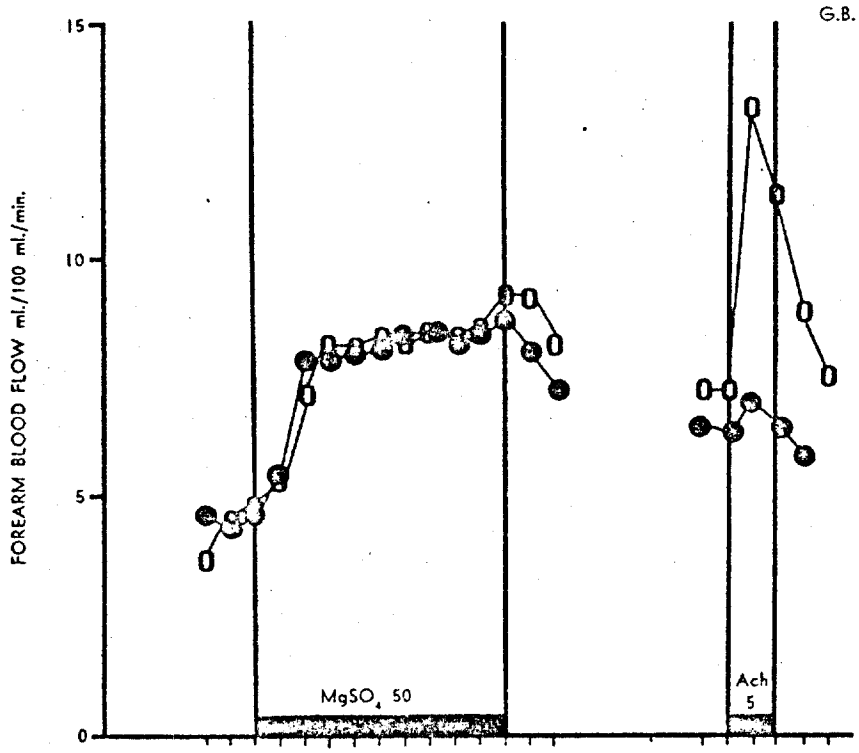


Fig. 4. The effect of hyoscine intra-arterially (0·1 mg./min. for 4 min.) on the response of forearm blood flow of 1 subject to magnesium sulphate (50 mg./min.). The frame to the right of the Fig. is the effect of acetylcholine (5 μg./min.) on forearm blood flow, ○, before and ●, after hyoscine.

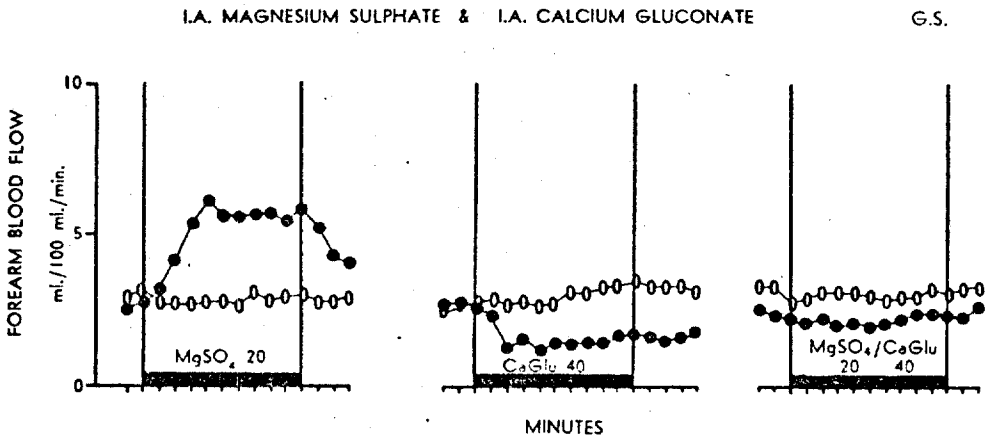


Fig. 5. The response of the blood flow through a normal forearm to intra-arterial magnesium sulphate, 20 mg./ min. (L-hand frame), calcium gluconate, 40 mg./min. (middle frame), and a mixture of magnesium sulphate 20 mg./min. and calcium gluconate 40 mg./min. (R-hand frame). ●, infused side; ○, control side.

salt and magnesium salt together. The resultant effect was a small fall in the flow. Similar results were obtained in the second experiment.

In Fig. 6 are shown the results of two experiments performed to study the effect of magnesium sulphate on the circulation through the skin and the muscle in the human forearm. A dose of 50 mg./min. increased the oxygen saturation of the effluent venous blood draining both the muscle and skin vascular beds.

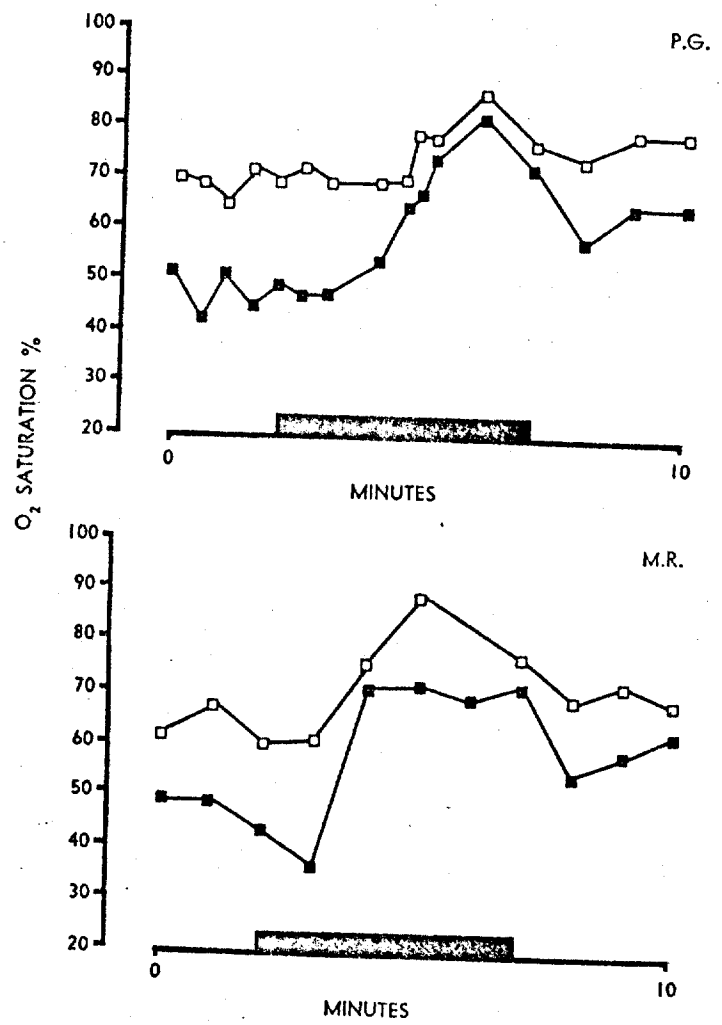


Fig. 6. The effect of an intra-arterial infusion of magnesium sulphate (50 mg./min.) into the brachial artery on superficial (□) and deep (■) venous oxygen saturation of the forearm of 2 subjects.

Fig. 7 portrays the effects of magnesium sulphate on the blood vessels of a surgically sympathectomised forearm. The frame to the right of the Figure shows the almost complete absence of constrictor effect of tyramine (an indirectly acting sympathomimetic amine) infused intra-arterially in a dose of 50 μg./min.

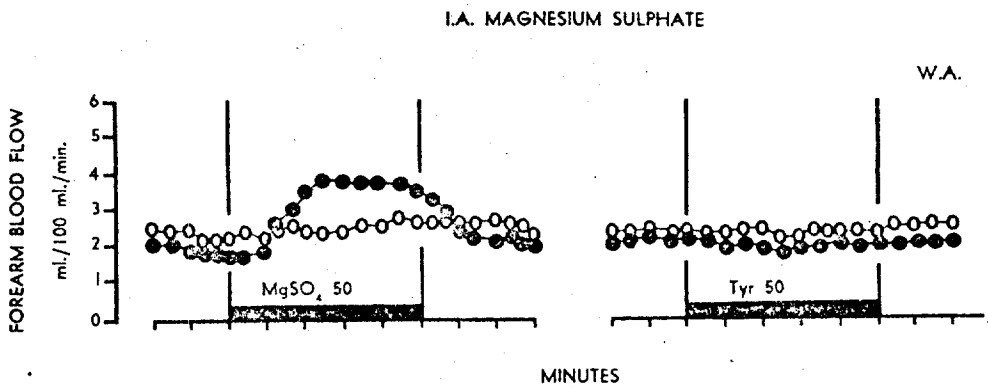


Fig. 7. The effect of intra-arterial magnesium sulphate (50 mg./min.) on blood flow in a sympathectomised forearm (L-hand frame) and that of intra-arterial tyramine (50 μg./min.) on the same vascular bed (R-hand frame).

which in a normal limb with intact sympathetic nerves would have produced a marked fall in forearm flow. However, magnesium sulphate produced a definite dilatation in the forearm of the usual order.

DISCUSSION.

The results of the foregoing experiments demonstrate that magnesium sulphate has a direct local vasodilator action on the blood vessels of the forearm in man. The vessels of both the skin and the muscle are affected. The response is not modified by administration of propranolol, hyoscine or mepyramine, indicating that the dilator action is not due to stimulation of β-receptors, to a cholinergic mechanism, nor to release of histamine. The local direct action on the blood vessels is further confirmed by the presence of a vasodilator action in the sympathectically denervated forearm.

These results extend the findings of Overbeck *et al.*. (1969) who reported that infusions of magnesium sulphate into the brachial artery caused an increase in forearm blood flow in man. On the basis of previous findings, Overbeck, Molnar and Haddy (1961) of an absence of a dilator action of the sulphate ion, they suggested that it was the magnesium ion that was responsible for the dilatation. They considered magnesium to be an endogenous vasodilator similar to bradykinin, histamine and acetylcholine.

Haddy (1960) has shown that infusions of sodium and magnesium salts into the brachial artery of the dog, at rates which raised the serum cation concentrations in the leg without significantly affecting concentrations in the body, caused vasodilatation, whereas calcium salts caused vasoconstriction. In our study the dilator action of magnesium sulphate was abolished when an equimolar dose of calcium gluconate was infused simultaneously into the brachial artery. When the calcium gluconate was administered by itself, it produced a considerable fall in forearm flow, and it would appear that the constrictor effect of this compound on the forearm vessels counteracted the dilator action of the magnesium salt when the two were infused together. However, the precise mechanism of this antagonistic action is not clear.

Acknowledgements. We are grateful to our colleagues and students who volunteered as subjects for these experiments. Support for this project was provided from a research grant of the National Health and Medical Research Council to Professor R. F. Whelan.

REFERENCES.

BERNSTEIN, M., and SIMKINS, S. (1939): 'The use of magnesium sulfate in the measurement of circulation time.' *Am. Heart J.*, 17, 218.

BLAIR, D. A., GLOVER, W. E., GREENFIELD, A. D. M., and RODDIE, I. C. (1959): 'Excitation of cholinergic vasodilator nerves to human skeletal muscles during emotional stress.' *J. Physiol.*, 148, 633.

BROWNE, S. E. (1964): 'Parenteral magnesium sulphate in arterial disease.' *The Practitioner*, 192, 791.

DUFF, R. S. (1952): 'Effect of sympathectomy on the response to adrenaline of the blood vessels of the skin in man.' *J. Physiol.*, 117, 415.

FREWIN, D. B., and WHELAN, R. F. (1968): 'The mechanism of action of tyramine on the blood vessels of the forearm in man.' *Br. J. Pharmac.*, 33, 105.

GREENFIELD, A. D. M. (1954): 'A simple water-filled plethysmograph for the hand or forearm with temperature control.' *J. Physiol.*, 123, 62P.

HADDY, F. J. (1960): 'Local effects of sodium, calcium and magnesium upon small and large blood vessels of the dog forelimb.' *Circulat. Res.*, 8, 57.

HAURY, V. G. (1939): 'The effect of intravenous injections of magnesium sulfate on the volume of the extremities.' *J. Lab. clin. Med.*, 24, 951.

MAYES, B. T. (1950): 'A textbook of obstetrics.' Australasian Publishing Company, Sydney, Wellington, London, p. 431.

MELTZER, S. J., and AUER, J. (1906): 'Physiological and pharmacological studies of magnesium salts. II. The toxicity of intravenous injections; in particular the effects upon the centres of the medulla oblongata.' *Am. J. Physiol.*, 15, 387.

OVERBECK, H. W., MOLNAR, J. I., and HADDY, F. J. (1961): 'Resistance to blood flow through the vascular bed of the dog forelimb. Local effects of sodium, potassium, calcium, magnesium, acetate, hypertonicity, and hypotonicity.' *Am. J. Cardiol.*, 8, 533.

OVERBECK, H. W., DAUGHERTY, R. M., and HADDY, F. J. (1969): 'Continuous infusion indicator dilution measurement of limb blood flow and vascular response to magnesium sulfate in normotensive and hypertensive men.' *J. clin. Invest.*, 48, 1944.

RODDIE, I. C., SHEPHERD, J. T., and WHELAN, R. F. (1956): 'Evidence from venous oxygen saturation measurements that the increase in forearm blood flow during body heating is confined to the skin.' *J. Physiol.*, 134, 444.

RODDIE, I. C., SHEPHERD, J. T., and WHELAN, R. F. (1957b): 'A spectrophotometric method for the rapid estimation of blood oxygen saturation, content and capacity.' *J. clin. Path.*, 10, 115.

SMITH, P. K., WINKLER, A. W., and HOFF, H. E. (1939): 'Electrocardiographic changes and concentration of magnesium in serum following intravenous injection of magnesium salts.' *Am. J. Physiol.*, 126, 720.

WINKLER, A. W., HOFF, H. E., and SMITH, P. K. (1940): 'Cardiovascular effects of potassium, calcium, magnesium and barium. An experimental study of toxicity and rationale of use in therapeutics.' *Yale J. Biol. Med.*, 13, 123.

American Journal of Clinical Nutrition
Vol. 20, No. 5, May, 1967, pp. 393-399
Printed in U.S.A.

Effect of Daily MgO and Vitamin B_6 Administration to Patients with Recurring Calcium Oxalate Kidney Stones[1,2]

Stanley N. Gershoff[3] and Edwin L. Prien[4]

Studies reported from this laboratory some years ago demonstrated that vitamin B_6 deficiency in rats and cats resulted in a marked increase in urinary oxalate (1, 2). This oxaluria was accompanied in cats by the formation of calcium oxalate renal stones similar in structure to those seen in humans and associated with secondary obstructive sequelae involving the lower urinary tract (3). The feeding of high levels of magnesium to vitamin B_6 deficient rats markedly reduced the formation of urinary stones, although the hyperoxaluria was not affected (4). At about the same time, it was shown (5) that renal calcium oxalate deposition in rats receiving 0.25% ethylene glycol in their drinking water could be completely prevented by diets high in magnesium and partially prevented by very high dietary levels of vitamin B_6. In view of these studies, it was decided to investigate the effects of daily oral administration of magnesium and vitamin B_6 on patients with histories of recurring calcium oxalate urolithiasis.

From Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts.

This work was supported in part by National Institute of Arthritis and Metabolic Diseases Grants-in-aid AM-03056 and AM 07725 and The Fund for Research and Teaching, Department of Nutrition, Harvard School of Public Health.

[3] Associate Professor of Nutrition. [4] Clinical Research Associate in Nutrition.

EXPERIMENTAL

Male and female adult patients who had made two or more calcium oxalate renal stones yearly during the 2 years prior to being approached were used in this study. For all of the subjects studied, crystallographic examination of their calculi had been made by one of us (E. Prien). Approximately 50% of the subjects studied were patients of Dr. Prien, the others were patients of cooperating urologists in various parts of the country. Because of the serious nature of their disease, all patients had been hospitalized, sometimes repeatedly for complete medical examination, prior to being accepted in this study. Patients with hyperparathyroidism, renal tubular acidosis, and other conditions commonly associated with recurrent calcigenous stone formation were excluded from the study. A few of the patients did have transient urinary infections but no other urinary tract lesions demonstrable by pyelography.

Before being started in this program, two 24-hr urine samples were obtained from all subjects. In addition, two blood samples, drawn at least 24 hr apart, were obtained from most of the patients from the Boston area. The patients were asked to take two tablets, each containing 100 mg MgO, and one tablet containing 10 mg pyridoxine daily. This treatment did not produce looseness of the bowels except in an occasional patient. In addition to the medication, all patients were instructed to avoid milk as a beverage but allowed the use of milk or cream in other foods. Intakes of cheese and other high calcium foods were restricted. They were asked to drink 2 qt of water per day. Most of the sub-

jects had been asked to observe these dietary precautions prior to the study as a consequence of their disease. The collection of blood and urine samples was repeated after the subjects had been on the experimental regimen for a year. Urines were analyzed for calcium (6), magnesium (7), citric acid (8), oxalic acid (9), xanthurenic acid (10), phosphate-P (11), and pyrophosphate-P (12).

In addition, the ability of the urines collected to hold oxalate in solution was determined by an adaptation of the method of Miller et al. (13). This method consisted of adjusting the pH of the urines studied to 6 and their calcium content to 15 mg/100 ml, and then filtering them. To 5 ml of the urines were then added varying amounts of 0.005 M sodium oxalate and water to a final volume of 7 ml. The amount of oxalate solution needed to give a reading of 10 on a Coleman nephelometer scale was considered an index of the ability of the urine to hold oxalate in solution.

Sera were analyzed for magnesium (7), lipoproteins (14), and total cholesterol and β-cholesterol (15). Serum fractions analyzed for β-cholesterol were obtained by preparative ultracentrifugation at a solution density of 1.063 g/ml (14).

RESULTS

Thirty-six patients have been maintained in this study for at least 5 years. In our own series of cases, 15 patients have now been in the program 5 years. There was no recurrence of kidney stone in nine. Two patients, each produced one stone in their 4th year in the program. Another passed several calculi over the Christmas holiday in his 1st year when he stopped taking the pills, one stone the 2nd year, and one stone in the 4th year. He had passed 11 calculi in the year before therapy was instituted and over 300 in the 14 years prior to entering this program. A fourth patient, a very busy executive of a large company, living a strenuous existence, passed one or two small calculi per year for 3 years, none since. A fifth patient with two existing small stones when the treatment was started showed no increase in their size for 2½ years, failed to come in for checkups after this period and stopped taking the drugs 6 months later. One and a half years later, one of the stones had grown considerably and caused symptoms resulting in an operation. One patient showed no improvement and continued to make stones.

In 21 cases maintained in the program for 5 years under the care of cooperating urologists, there was no stone recurrence or marked improvement (decreasing stone frequency) in 16. Some difficulty was encountered in determining exactly whether stones passed were newly formed stones or were pre-existing calculi because several of the patients had a number of calculi existing in their kidneys when treatment was started. Also, there was a question as to whether a stone had really been passed in several instances when symptoms occurred and no stone was recovered. Kidney-ureter-bladder films provided the only objective data in these cases. In five subjects there was failure. New stones were formed or existing stones grew larger. One of these patients kept on forming and passing calculi.

Table 1 shows the effects on urine and serum constituents of being in this program 1 year. These data indicate that the treatment used significantly raised urinary calcium and citric acid, and lowered urinary xanthurenic acid and serum magnesium. Urinary oxalic acid, magnesium, phosphate-P and pyrophosphate-P, and serum total and β-cholesterol were not significantly changed. Serum lipoprotein values were also obtained by ultracentrifuge analysis. There was a significant lowering in the S_f 12–20 and S_f 20–35 fraction, but not in the other lipoproteins measured.

Using the procedure described for measuring the ability of urines to hold calcium oxalate in solution, the average amount of 0.005 M sodium oxalate solution needed to reach the end point for 51 control urines was 1.3 ml. After a year, the urines of 45 of the 51 patients studied showed an in-

creased ability to hold oxalate in solution. Of these, 31 had such an increased capacity to hold oxalate in solution that 2 ml of oxalate solution, the maximum used in our procedure, was not sufficient to reach the turbidimetric end point.

DISCUSSION

During the early part of this century, there was considerable interest in using magnesium salts in the treatment of urinary stone disease. Until recently, interest in the treatment and prevention of calcigenous stones by magnesium administration was mainly sustained by the work of Hammarsten. In 1929 she reported that the solubility of calcium oxalate in water was markedly increased by $MgCl_2$ (16). She also reported that she was able to prevent the formation of calcium oxalate urinary stones in rats by adding magnesium to their diets and that dietary magnesium decreased oxalate excretion in rats and human subjects (17).

De Albuquerque and Tuma (18) have also reported that the feeding of MgO to oxalate stone formers reduced their excretion of oxalic acid. In the present study, oxalate excretion was not significantly affected in a group of 50 patients receiving MgO and vitamin B_6 therapy daily for a year. On the basis of a large number of studies done in this laboratory, we would interpret any lowering of oxalate excretion by vitamin B_6 and MgO treatment as being related to the administration of vitamin B_6. It has been clearly shown in rats (1) and cats (2) that vitamin B_6 deficiency is accompanied by an increase in oxalate excretion. In unpublished studies, similar results have been obtained in rhesus and Cebus monkeys. In two studies done in a mental hospital on subjects receiving a common diet considered to be adequate in vitamin B_6, the administration of vitamin B_6 was accompanied by a marked decrease in oxalate excretion (19). An association between oxaluria and high urinary xanthurenic acid and low vitamin B_6 excretion levels in Burmese subjects has been suggested (20), and oxalate excretion has been reported as being significantly higher in poorly nourished Thai village children than in private school children in a nearby city (21).

TABLE 1

Effect of Daily MgO and Vitamin B_6 Administration on Urinary and Serum Constituents of Patients with Recurring Calcium Oxalate Renal Calculi

	No. of Patients	Control	After 1 Year of Treatment	SE of Mean Differences
Urinary Constituents, mg/24 hr				
Calcium	51	218.0	276.0	13.0[b]
Magnesium	51	79.0	87.0	8.0
Citric acid	51	435.0	531.0	33.0[a]
Oxalic acid	50	33.9	34.5	2.3
Xanthurenic acid	51	12.3	5.1	1.2[b]
Phosphate-P	25	849.0	821.0	87.0
Pyrophosphate-P	25	1.31	1.24	0.19
Serum constituents, mg/100 ml				
Magnesium	25	2.18	1.88	0.05[b]
Total cholesterol	25	277.0	262.0	10.0
β-Cholesterol	25	131.0	136.0	12.0
Lipoproteins				
S_f 0–11	25	333.0	345.0	14.0
S_f 12–20	25	54.0	32.0	6.6[a]
S_f 21–35	25	31.0	20.0	3.5[a]
S_f 36–100	25	62.0	55.0	11.0
S_f 101–400	25	21.0	11.0	4.9

[a] $P < 0.01$.
[b] $P < 0.001$.

Extensive studies on rats in this laboratory have never indicated that dietary magnesium affects oxalate excretion, although it protects against the deposition of calcium oxalate in the kidneys of vitamin B_6-deficient rats (4) or rats receiving ethylene glycol in their drinking water (5). The use of radioactive precursors of oxalate in rats supports the conclusion that their conversion to oxalate is probably not affected by dietary magnesium but is quite sensitive to dietary vitamin B_6 levels. Thus it would

appear that the role of magnesium in preventing calcium oxalate deposition in the rat urinary tract is not the result of an effect on the amount of oxalate excreted. This protective effect is obtained not only without a decrease in urinary oxalate but may be associated with an increase in urinary calcium (22). Increased urinary calcium following magnesium administration in animals and man has commonly been observed. In the present work, the groups of subjects studied excreted approximately 25% more calcium after receiving MgO and vitamin B_6 for a year.

A possible rationale for an effect of MgO and vitamin B_6 administration in protecting against calcium oxalate urolithiasis might involve an effect on the solvent characteristics of urine. Vermeulen et al. (23) and Miller et al. (24) have reported that a variety of metabolites found in urine are effective in increasing the solubility of calcium phosphate and calcium oxalate in water. Citrate and magnesium are particularly effective as solubilizers. Mukai and Howard (25) have reported that urine from stone formers, which calcified rachitic rat cartilage, becomes noncalcifying when magnesium is added to it. Du Mont and Nowak (26) found that the drinking of mineral water with a high magnesium content increased the colloid stability of urine from subjects with and without urinary stone disease. In previous studies from this laboratory it was observed that calcium oxalate stone patients with no history of urinary tract infection excreted less citric acid than normal subjects. Furthermore, vitamin B_6-deficient rats, which form calcium oxalate urinary calculi, excrete only a small fraction of the citric acid excreted by control rats. This decrease in citric acid was prevented by feeding vitamin B_6 or high dietary levels of magnesium (4).

The results of the present studies indicate that the capacity of the urines of 45 of 51 subjects studied to hold calcium oxalate in solution was increased by the experimental regimen. This increase was of sufficient magnitude so that with the method used for measuring the ability of urine to maintain calcium oxalate in solution, we were unable to measure calcium oxalate turbidity in the urines of 31 of the subjects after a year on the experiment. It seems unlikely that the change in the solvent characteristics of the urine from the patients studied can be explained simply on the basis of altered magnesium and citric acid content. There was not a statistically significant rise in urinary magnesium as a result of the treatment used. It is unfortunate that we were unable to determine the distribution of urinary magnesium in bound and ionic forms. The treatment used resulted in a significant increase in average urinary citric acid of about 100 mg/day. This would not entirely explain the increased solubility of calcium oxalate in the urine of the subjects. The excretion of pyrophosphate, which has been reported as being extremely active in inhibiting the formation of calcium deposits in vivo and in vitro (27), was not altered in the 25 subjects studied.

There have now been 36 patients with histories of recurring calcium oxalate kidney calculi who have received daily treatment with MgO and vitamin B_6 for at least 5 years. The problems in carrying on this type of experiment are often disheartening. Individuals with urinary stone disease often endure a great amount of pain. It is easy to enlist the cooperation of patients who have recently had an attack of stone disease. However, after a year or two without a further attack, their interest wavers and their cooperation becomes increasingly poor. Since in most individuals who form stones, calculi may only appear sporadically, any stone-prevention program must be evaluated over a long period of time. In this report we have not included clinical data on approximately 50 patients who dropped out of the study after 1–3 years, mostly because of their having no

gather trouble. It is impossible to maintain an accurate check on subjects to know whether they are following instructions. One must, to a great extent, depend on their word. Two subjects who continued to form stones after being put in this program later admitted that they had not taken their pills.

We are encouraged by the results obtained so far. Of 36 patients who have been maintained in this program for 5 years or more, 30 have shown no recurrence or decreased recurrence of stone formation. We recognize that only a much larger series of cases treated over a long period of time will determine the efficacy of this regimen.

There is no reason to believe that there is a common etiology in all cases of calcium oxalate urolithiasis. Therefore, it is not unreasonable that a prophylactic treatment might be extremely effective in some patients but not in others. Some people, instead of forming small numbers of large calcium oxalate stones, form large numbers of small stones which they pass. Because of the number of attacks they suffer, it is relatively easy to evaluate prophylactic measures for them. We have already in this report described such a case, a man who passed over 300 stones in the 14 years before entering this program, who now only passes an occasional small stone. Moore and Bunce (28) have recently reported two cases of individuals of this nature who passed 2–4 calculi/month. Oral administration of 420 mg of MgO/day protected both of them without any adverse reactions to the MgO. Cessation of therapy in one of these individuals for a month resulted in resumption of calculus formation.

Malkiel-Shapiro and Bersohn (29) have reported a dramatic clinical improvement in many cases of coronary heart disease following parenteral administration of $MgSO_4$, accompanied by reversion of abnormal serum lipoprotein values to normal. Parsons et al. (30) observed a similar effect of intramuscular $MgSO_4$ on patients with coronary heart disease associated with a marked reduction in serum cholesterol and β-lipoproteins. In rats (31) and monkeys (32) fed diets containing cholesterol, magnesium deficiency has been associated with the production of atherosclerosis, although only in the monkeys did the magnesium content of the diet affect the serum cholesterol levels. Vitamin B_6 deficiency has also been associated with the production of atherosclerosis in monkeys and dogs, and vitamin B_6-deficient monkeys and chicks show a greater degree of hypercholesteremia when fed cholesterol than control animals (33). In view of these observations, serum magnesium, cholesterol, and lipoproteins were determined in the present study on 25 subjects. The daily ingestion of MgO and vitamin B_6 for a year did not affect serum cholesterol values but resulted in a decrease in serum Mg and β-lipoproteins above S_f 12, particularly in the S_f 12–35 groups. Just what the serum changes observed in the present study mean is obscure. The reduction in serum lipoproteins is of some interest in that it may be relevant to the observations cited above.

SUMMARY

Patients with histories of recurring calcium oxalate renal stone formation have been given daily oral supplements of 200 mg of MgO and 10 mg of pyridoxine for extended periods of time. Thirty of 36 patients maintained on this program for 5 years or more have shown no recurrence or decreased recurrence of stone formation. After 1 year on this regimen, urines obtained from 51 patients showed a marked increase in their capacity to maintain calcium oxalate in solution, significantly raised calcium and citric acid levels, and decreased xanthurenic acid levels. No significant changes were observed in urinary magnesium, oxalic acid, phosphate-P or pyrophosphate-P values. Decreases in serum β-lipoproteins were also observed in a group of 25 of these subjects.

The authors wish to thank Miss Alice Carney, Mrs. Aurora Chung and Mrs. Judith Hershenson for their technical assistance. We should also like to thank the following urologists who have cooperated with us in this study: Drs. D. Atherton, B. Bloom, R. H. Bradley, Jr., H. A. Buchtel, J. L. Campbell, C. J. Cooney, J. W. Dworin, J. L. Fischman, P. C. Funk, W. L. Geissler, P. L. Getzoff, W. W. Hoffman, A. Johnston, T. Latiolais, J. G. Menville, K. Mooney, A. E. Mycbrott, O. Myers, A. J. Oliker, L. F. Pfeifer, E. C. St. Martin, H. M. Spence, B. E. Trichel, and H. M. Yearwood. The pyridoxine and MO tablets used were kindly provided by the Upjohn Co., Kalamazoo, Michigan.

REFERENCES

1. Gershoff, S. N., and F. F. Faragalla. Endogenous oxalate synthesis and glycine, serine, deoxypyridoxine interrelationships in vitamin B_6-deficient rats. *J. Biol. Chem.* 234: 2391, 1959.
2. Gershoff, S. N., F. F. Faragalla, D. A. Nelson and S. B. Andrus. Vitamin B_6 deficiency and oxalate nephrocalcinosis in the cat. *Am. J. Med.* 27: 72, 1959.
3. Andrus, S. B., S. N. Gershoff, F. F. Faragalla and E. L. Prien. Production of calcium oxalate renal calculi in vitamin B_6-deficient rats. Study of the influence of urine pH. *Lab. Invest.* 9: 7, 1960.
4. Gershoff, S. N., and S. B. Andrus. Dietary magnesium, calcium and vitamin B_6 and experimental nephropathies in rats: calcium oxalate calculi, apatite nephrocalcinosis. *J. Nutr.* 73: 308, 1961.
5. Gershoff, S. N., and S. B. Andrus. Effect of vitamin B_6 and magnesium on renal deposition of calcium oxalate induced by ethylene glycol administration. *Proc. Soc. Exptl. Biol. Med.* 109: 99, 1962.
6. Sobel, A. E., and B. A. Sobel. The determination of calcium in urine. *J. Lab. Clin. Med.* 26: 585, 1940.
7. Schachter, D. The fluorometric estimation of magnesium in serum and urine. *J. Lab. Clin. Med.* 54: 763, 1959.
8. McArdle, B. A modified method for the microdetermination of citric acid. *Biochem. J.* 60: 647, 1955.
9. Powers, H. H., and P. Levatin. A method for the determination of oxalic acid in urine. *J. Biol. Chem.* 154: 207, 1944.
10. Glazer, H. S., J. F. Mueller, C. Thompson, V. R. Hawkins and R. W. Vilter. A study of urinary excretion of xanthurenic acid and other tryptophan metabolites in human beings with pyridoxine deficiency induced by desoxypyridoxine. *Arch. Biochem. Biophys.* 33: 243, 1951.
11. Fiske, C. H., and Y. Subbarow. The colorimetric determination of phosphorus. *J. Biol. Chem.* 66: 375, 1925.
12. Fleisch, H., S. Bisaz, and A. D. Care. Effect of orthophosphate on urinary pyrophosphate excretion and prevention of urolithiasis. *Lancet* 1: 1065, 1964.
13. Miller, G. H., C. W. Vermeulen and J. D. Moore. Calcium oxalate solubility in urine: experimental urolithiasis XIV. *J. Urol.* 79: 607, 1958.
14. DeLalla, O. F., and J. W. Gofman. Ultracentrifugal analysis of serum lipoproteins. *Methods Biochem. Anal.* 1: 459, 1954.
15. Carpenter, K. J., A. Gotsis and D. M. Hegsted. Estimation of total cholesterol in serum by a micro method. *Clin. Chem.* 3: 233, 19[illegible].
16. Hammarsten, G. On calcium oxalate and its solubility in the presence of inorganic salts with special reference to the occurrence of oxaluria. *Compt. Rend. Trav. Lab. Carlsberg* 17: 1, 1929.
17. Hammarsten, G. *Etiologic Factors in Renal Lithiasis*, edited by A. J. Butt. Springfield, Ill.: Thomas, 1956.
18. De Albuquerque, P. F., and M. Tuma. Investigations on urolithiasis, II. Studies on oxalate. *J. Urol.* 87: 504, 1962.
19. Gershoff, S. N., A. L. Mayer and L. L. Kulczycki. Effect of pyridoxine administration on the urinary excretion of oxalic acid, pyridoxine and related compounds in mongoloids and nonmongoloids. *Am. J. Clin. Nutr.* 7: 76, 1959.
20. Nutrition Survey of Burma. May 1963. A report by the Interdepartmental Committee on Nutrition for National Defense.
21. Gershoff, S. N., E. L. Prien and A. Chandrapanond. Urinary stones in Thailand. *J. Urol.* 90: 285, 1963.
22. Faragalla, F. F., and S. N. Gershoff. Interrelations among magnesium, vitamin B_6, sulfur and phosphorus in the formation of kidney stones in the rat. *J. Nutr.* 81: 60, 1963.
23. Vermeulen, C. W., E. S. Lyon and G. H. Miller. Calcium phosphate solubility in urine as measured by a precipitation test: experimental urolithiasis XIII. *J. Urol.* 79: 596, 1958.
24. Miller, G. H., C. W. Vermeulen and J. D. Moore. Calcium oxalate solubility in urine: experimental urolithiasis XIV. *J. Urol.* 79: 607, 1958.
25. Mukai, T., and J. E. Howard. Some observations on the calcification of rachitic cartilage by urine. One difference between "good" and "evil" urines, dependent upon content of magnesium. *Bull. Johns Hopkins Hosp.* 112: 279, 1963.
26. Du Mont, H. L., and P. Nowak. Überlegungen und Untersuchungen zur Frage der Harnsteinprophylaxe: Magnesium. *Z. Urol.* 58: 1, 1964.
27. Fleisch, H., and S. Bisaz. Isolation from urine of

pyrophosphate, a calcification inhibitor. *Am. J. Physiol.* 203: 671, 1962.

28. Moore, C. A., and G. E. Bunce. Reduction in frequency of renal calculus formation by oral magnesium administration. *Invest. Urol.* 2: 7, 1964.

29. Malkiel-Shapiro, B., and I. Bersohn. Parenteral magnesium sulfate therapy in coronary heart disease. *Med. Proc.* 2: 455, 1956.

30. Parsons, R. S., T. Butler and E. P. Sellars. The treatment of coronary artery disease with parenteral magnesium sulfate. *Med. Proc.* 5: 487, 1959.

31. Vitale, J. J., P. L. White, M. Nakamura, D. M. Hegsted, N. Zamcheck and E. E. Hellerstein. Interrelationships between experimental hypercholesteremia, magnesium requirement, and experimental atherosclerosis. *J. Exptl. Med.* 106: 757, 1957.

32. Vitale, J. J., H. Vellez, C. Guzman and P. Correa. Magnesium deficiency in the Cebus monkey. *Circulation Res.* 12: 642, 1963.

33. Greenberg, L. D. Atheriosclerotic, dental and hepatic lesions in pyridoxine-deficient monkeys. *Vitamins Hormones* 22: 677, 1964.

Metabolism, 9: 646-659 (1960)

Gastrointestinal Absorption and Excretion of Mg^{28} in Man

By L. A. Graham, J. J. Caesar and A. S. V. Burgen

Absorption of Mg^{28} after oral administration to 10 patients and 3 normal subjects was studied by fecal collection and by a method based on analysis of plasma radioactivity. On an average diet (20 mEq. of Mg/day), 44.3 per cent of the ingested radioisotope was absorbed. On a low Mg diet (1.9 mEq./day), absorption was 75.8 per cent; whereas on a high Mg diet (47 mEq./day), it was depressed to 23.7 per cent. Absorption began within an hour of ingestion and continued at a steady rate from 2 to 8 hours. Little absorption took place after 12 hours. This pattern is consistent with a fairly uniform absorption throughout the small intestine and little or no absorption from the large bowel. The findings suggest that absorption is influenced by the load presented to the intestinal mucosa, but is not linearly related to the load. Absorption does not appear to be importantly affected by depletion or repletion of body magnesium, at least when carried out over a 5 day period in which changes in serum magnesium and urinary clearance occurred.

STUDIES on the gastrointestinal handling of magnesium in normal human subjects have been carried on for many years. Magnesium analysis, particularly of stool and foodstuff by current methods, is inaccurate, as attested by the large number of analytic methods and modifications recorded in the literature. The accuracy of recorded values for magnesium requirements, daily intake, and fecal magnesium is therefore dubious.

Magnesium occurs widely throughout nature and is abundant in most foods. It is present in chlorophyll in a position analogous to that of iron in hemoglobin. Analysis of magnesium intakes of a large population by Duckworth and Warnock[1] suggested that 10 per cent of the subjects received less than 20 mEq./day. A high proportion of daily magnesium was supplied by cereals and dairy products. These and other authors[2] suggest that about 20 mEq./day of magnesium is required for balance, and this corresponds to the minimal values usually given for daily magnesium intake[3] although Sherman[4] quotes a normal range of 11 to 54 mEq./day. Tibbetts and Aub[5] report a positive balance on a diet of 18.3 mEq./day but suggest the requirement may be less.

That the daily requirements *are* much less is suggested by studies using magnesium-deficient diets. Two subjects were reported by Fitzgerald and Fourman[6] to have had no ill effects after three weeks on a diet of 1.1 mEq. Mg./per day. Net magnesium losses were 46 and 72 mEq., respectively, with the dietary deficiency of the latter subject being compounded by an ion exchange resin. Balance studies performed by Barnes, Cope and Harrison[7] on

From the McGill University Clinic, Montreal General Hospital, Montreal, Canada.
Supported by grants from the Cancer Research Society, Inc. and the Cooper Fund of McGill University. We wish to acknowledge the valuable help given by Miss J. Robb, Miss M. White and Mrs. C. Lash as well as our three experimental subjects in the course of this work.

Received for publication Aug. 10, 1959.

four subjects receiving a daily intake of less than 0.12 mEq./Mg./per day over a 10 day period showed a marked conservation of magnesium, calculated from urinary and fecal excretion data. The latter authors suggest that after an initial period of adjustment to a deficient diet magnesium balance can be maintained on as little as 1 mEq. per day.

With the recent availability of a radioactive isotope of magnesium, Mg^{28}, the study of magnesium metabolism by tracer technic has become possible. Most of the reports to date concern the parenteral administration of the isotope to determine magnesium space and tissue distribution of magnesium.

Fecal recovery of Mg^{28} following intravenous injection of the isotope provides data on the rate of excretion of body magnesium by the gut. Zumoff[8] and his co-workers were unable to detect significant activity following infusion. Injection of three control subjects in our own laboratory was followed by a daily fecal excretion rate of 0.45 to 1.08 per cent.[9] Values for resecretion in the older literature are too dependent on doubtful analytic methods to be quantitatively accurate, although all suggest a negligible amount of body magnesium excreted by the feces.

Orally administered Mg^{28} has been recovered from stool, urine, and plasma by Aikawa et al.,[10] and Zumoff[8] and his co-workers. These authors found a fecal excretion of 50 to 90 per cent of the orally administered dose for subjects on regular dietary intakes of magnesium. These values correspond to 60 per cent fecal excretions of stable magnesium reported by Shohl[11] and Flink.[2] Dempsey[12] and her co-workers found fecal excretion values of 33 to 96 per cent of the ingested magnesium in the cases of 19 subjects on intakes ranging from 12.9 to 30.9 mEq. per day, and concluded that there was no relation between magnesium intake and fecal excretion of magnesium in these subjects.

There is as yet no recorded information concerning the site and mechanism of magnesium absorption. Experiments on rabbits by Aikawa[13] suggest that no absorption was similar in starved animals in whom the unabsorbed magnesium remained in the large intestine for three days as compared with fed animals who evacuated feces normally. There have been no experiments similar to those reported by Schacter,[14] who studied the site of absorption of Ca^{45} by means of isolated gastrointestinal loops.

The purpose of the present experiments was to obtain further information on the effect of variation of dietary intake on magnesium absorption and excretion in the human subject using Mg^{28}. Normal values for fecal and urinary excretion by control subjects on regular diets will be presented, as well as the effect on these functions obtained by varying the dietary magnesium. The approximate time course of absorption has been followed by an analysis of the plasma radioactivity.

Materials and Methods

Subject Selection

Subjects for control experiments were chosen from the general medical and surgical wards. All were believed to have normal gastrointestinal function, including normal appetite

Table 1.—*Control Subjects*

Subject	Age	Diagnosis	Serum Mg (mEq./L.)	*Intake Mg (mEq./day)	96 hour fecal excretion Mg^{28} (%)	Magnesium absorption (mEq./day)	Urine excretion % absorbed Mg^{28} in 48 hr.	"Resecretion" (%/dose/day)
H. E.	49	myocardial infarct	1.92	26.5	35.2	17.2	15.1	0.7
D. E.	55	myocardial infarct	1.92	18.3	67.5	5.9	8.9	2.3
J. W.	42	myocardial infarct	2.07	25.4	58.4	10.6	9.5	0.8
C. H.	39	rheumatic heart disease	2.31	20.5	40.7	12.2	7.6	1.3
F. C.	51	rheumatic heart disease	2.44	18.7	74.1	4.8	3.5	1.8
E. G.	25	rheumatic heart disease	1.98	21.2	74.6	5.4	11.0	1.8
W. B.	59	coronary insufficiency	2.06	29.6	53.6	9.6	3.3	
A. D.	58	myocardial infarct	2.34	20.4	57.5	8.7	7.2	
R. W.	64	myocardial infarct	2.12	20.4	48.0	10.6	7.7	
J. G.	28	fractured femurs	1.83	14.8	47.3	7.8	8.9	0.35
Mean ± S.D.			2.10±0.20	20.7±3.3	55.7±13.4	9.3±3.7	8.4±3.5	1.4±0.6

and regular bowel movements. Choice was limited because the patients were required to eat the standard diets for about ten days, and because cooperation was essential. Patients with heart disease were in general most satisfactory. Serum calcium, phosphorus, potassium, and protein electrophoresis were normal in all cases. Thus in table 1 nine out of ten subjects suffered from some form of heart ailment, those with coronary artery disease usually being in the convalescent stage of myocardial infarction, those with rheumatic heart disease being in hospital for assessment regarding surgical treatment or for regulation of drug therapy. The ages ranged from 25 to 64 years. There were eight males and two females.

Subjects on abnormal diets consisted of three young adult volunteers. All were associated with the hospital and interested in the experiment. All were in good health. Age, weight, height for each were as follows: Miss M. C.: 28 yrs., 58 Kg, 157.5 cm.; Mr. F. W.: 24 yrs., 92.3 Kg., 190.5 cm.; Mr. D.J.: 26 yrs., 60.2 Kg., 170 cm.

Administration of Isotope

Mg^{28} was obtained from Brookhaven as $MgCl_2$ the specific activity at the time of using being about 5 μc./mEq. The $MgCl_2$ was taken almost to dryness and then diluted with 0.9 per cent NaCl to a concentration of 1 μc./ml. Five ml. aliquots were pipetted into milk or fruit juice. The subject sipped the drink throughout breakfast. Aliquots were set aside for preparation of standards.

Diet

Diets containing varying amounts of magnesium were prepared from the table values.[4,15] Control diets of about 20 mEq. Mg/day were prepared according to the patient's taste, but were unchanged throughout the test period. Diets supplemented to about 50 mEq./day consisted of the basic control diet to which were added 30 mEq. of magnesium (as magnesium acetate) evenly divided in the three meals. Diet low in magnesium were prepared by the method of Barnes, Eddision and Cope,[16] using "Pream"* from which the magnesium had been removed by passage through a cation exchange resin† column. A diet containing less than 2 mEq. Mg/day was used for depletion studies and consisted of: "Pream" (1200 ml./day); "Pream" products such as ice cream (135 Gm.) and tapioca (260 Gm.); shortbread (30 Gm.); apple sauce (60 Gm.); fresh cranberries (35 Gm.); corn flakes (20 Gm.); sugar (75 Gm.); lemonade (400 cc.); and tea (4 Gm. dry). Six

*"Pream" from the M & R Dietetic Laboratories, Inc., Columbus, Ohio, U.S.A.
†Dowex 50-X4 from the Dow Chemical Company, Midland, Mich., U.S.A.

Table 2.—*Effect of Dietary Magnesium on Absorption of Magnesium*

Subject	Mg Intake (mEq./day)	Serum Mg (mEq./L.)	Fecal Mg^{28} excretion % in 96 hr.	Magnesium absorption (mEq./day)	Urine excretion % absorbed Mg^{28} in 48 hr.	Renal clearance (ml./min.)
F. W.	2.8	1.99	29.5	1.97	3.4	1.08
D. J.	2.9	1.94	21.4	2.24	2.4	0.80
M. C.	2.9	2.23	21.7	2.24	4.7	0.88
Mean	2.9	2.05	24.2	2.15	3.5	0.92
F. W.	47.8	2.21	76.8	11.1	9.6	2.79
D. J.	52.7	2.40	73.0	14.2	16.0	3.23
M. C.	43.1	2.42	79.1	9.1	28.8	3.24
Mean	47.8	2.34	76.3	11.4	18.1	3.09
Control (10)	20.7 ± 3.3	2.1 ± 0.2	55.7 ± 13.4	9.3 ± 3.7	8.4 ± 3.5	2.29

mEq. diets were prepared by adding certain vegetables and an egg to the above diet. Mg^{28} recovery experiments on "Pream" before and after passage through the ion exchange column showed more than 95 per cent removal of the isotope.

Chemical analyses were performed on aliquots of many of the diets. In all cases the analyses values were less than the standard table values for magnesium, the mean ratio of analysed to table values being 82.6 ± 15 per cent. Recoveries ranged from 80 to 140 per cent. Because of the incompleteness of these data and the inaccuracy of the technic, recorded values for magnesium are from standard tables, which allow for consistency for the various diets.[4,17] Values for calcium, phosphorus, protein, and caloric intake are also taken from standard tables of food values. The standard diet contained Mg, 20.7 mEq.; Ca, 723 mg.; P, 1139 mg.; protein, 74.2 grams; Calories, 1974. The low diet contained Mg, 1.9 mEq.*; Ca, 686 mg.; P, 947 mg.; protein, 18.1 grams; Calories, 1957. The supplemented diet Mg, 47.8 mEq.; Ca, 802 mg.; P, 1308 mg.; protein, 85 grams, and Calories, 1741.

Balance Technic and Specimen Collection

Patients on measured diets were placed on a 3 to 4 day equilibration period with a carmine marker given immediately before the isotope meal (breakfast). The test diet was continued throughout the period of stool collections. To ensure adequate bowel function methyl cellulose was administered as needed. As a non-irritant bulk-producing agent this laxative was felt to be unlikely to interfere with gastrointestinal handling of magnesium. Stool collection began following administration of the isotope and was continued for 96 hours. Twenty-four hour urine collections began on the first day of the diet and continued throughout the balance, with more frequent samples being obtained during the 12 hour period immediately following administration of the isotope. Plasma magnesium determinations were performed at the beginning of the experiment and following isotope administration.

Studies involving dietary variations were performed on three young adult volunteers. Diets of about 2 mEq. were continued for five days, an oral Mg^{28} absorption study being performed on the fifth day. Twenty-four hours after administration of the isotope the subjects were allowed a diet of 6 mEq./day for the remainder of the collection period. This did not interfere with absorption data, but was low enough in magnesium to minimize erroneous Mg^{28} excretion in the urine due to a "flushing" effect. After a 4 day intermission the subjects were placed on the supplemented magnesium diet (about 50 mEq./day) for a 10 day period, with a second Mg^{28} absorption study being performed on the sixth day.

*Subjects receiving low magnesium diets were equilibrated on a diet averaging 1.9 mEq./day. Since the isotope contained stable magnesium carrier, calculations based on magnesium load required that the amount of carrier be added to the daily intake, the mean value then being 2.9 mEq./day (table 2).

Collection of Specimens

Whole blood was collected by venipuncture, transferred to heparinized tubes, and plasma separated immediately for magnesium determination. The time of collection did not influence plasma level, as shown by analysis of 24 individual determinations at various intervals following normal meals. Five ml. aliquots of plasma were pipetted into glass counting tubes and counted in a well-type scintillation counter. Voided urine specimens were acidified to pH 6 with glacial acetic acid before aliquots were taken for chemistry. Counting specimens consisted of 100 ml. samples in glass screw-capped bottles. Stool specimens were collected in plastic bags, dry ashed, transferred to glass counting jars, and made up to 50 ml. volumes with melted gelatin and then cooled to solidify the sample. The gelatin maintained the ash in a relatively homogeneous suspension, thus assuring constant geometry. Following counting, the specimens were ashed, dissolved in concentrated HCl, titrated to pH 4 (methyl red) with concentrated NH_4OH and diluted HCl, and made up to 100 ml. volumes. Aliquots of diet homogenate were similarly ashed, dissolved in concentrated HCl and titrated. All aliquots of urine, stool and diet for chemical analysis were diluted to concentrations of about 2 mEq./L.

Chemical Methods

Magnesium analysis was performed by the titan yellow method, modified from Andreason[18] as follows: To the 3 ml. aliquot of centrifugate, 1 ml. of 0.15 per cent polyvinyl alcohol, 3 ml. of 0.02 per cent titan yellow (alcohol used as stock preservative) and 2 ml. 4N NaOH were added. A 2 cm. cuvette was used in the Beckman model "B" spectrophotometer. Standard deviation of 43 analyses of pooled control serum was ± 5.24 per cent. Serum magnesium of 57 control subjects was 2.15 ± 0.20 mEq./L. Normal 24 hour urine values range from 4 to 10 mEq./day. Recoveries on urine and serum were from 95 to 105 per cent. Serum calcium, phosphorus, potassium, and protein electrophoresis were performed by routine laboratory methods.

Subject Standardization

All subjects maintained a steady weight throughout the duration of the experiment. All fecal and urinary data are presented in relation to the individual without correction for variation in body size. All plasma Mg^{28} concentrations and data derived from them have been standardized to a 70 kilo man, as have values for renal clearance.

Results

Fecal and Urinary Excretion

The cumulative fecal and urinary excretion of Mg^{28} in a normal subject on an intake of 23.7 mEq. magnesium per day is shown in figure 1. The bulk of the unabsorbed isotope had appeared in the feces by 36 hours and following this there was only a small further loss. This late excretion may represent intestinal contents that are lagging behind the main bulk of isotope but it could also be explained quite readily on the basis of re-excretion into the bowel of absorbed isotope. The rate of slow excretion in this subject was approximately 2 per cent of the absorbed isotope per day. When the same subject was given Mg^{28} intravenously the average fecal excretion was 1.1 per cent of the retained isotope per day.

The curves in figure 1 are representative of the values found in subjects on normal diet. In this case 58.4 per cent of the administered dose had been recovered in the feces in 96 hours corresponding to an absorption of 41.6 per cent of the dose. In the first 48 hours 4.1 per cent of the administered dose

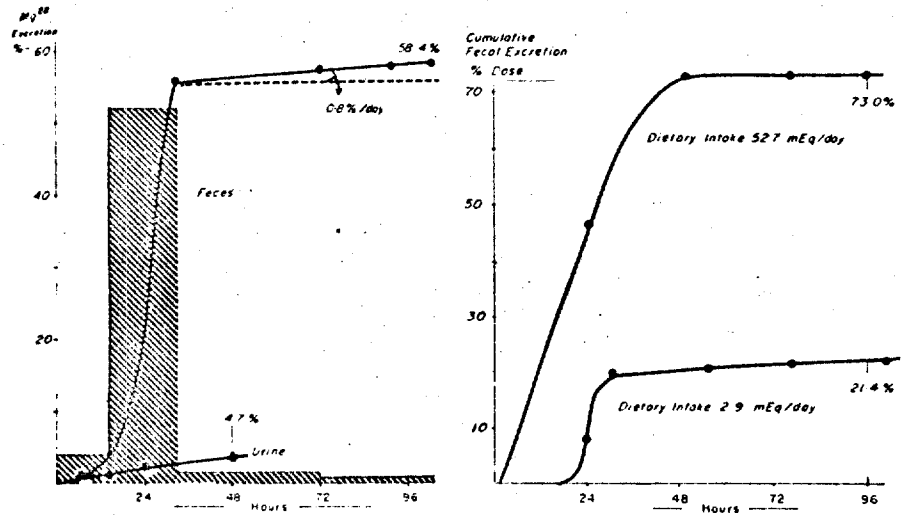


Fig. 1. *(left)*—Fecal and urinary excretion in a normal subject on a dietary intake of 23.7 mEq. Mg./day. Mg^{28} content of individual stools are shown by hatched areas. ●——●, cumulative fecal output; ★——★, cumulative urinary output. Ordinate: Mg^{28} as per cent of administered dose.

Fig. 2.—Cumulative fecal excretion of Mg^{28} in the same subject on a low (2.9 mEq./day) and high (52.7 mEq./day) dietary intake.

(9.4 per cent of the absorbed amount) was excreted in the urine. In the same individual after intravenous administration 11.4 per cent of the dose was passed in the first 48 hours.

Although in this subject unabsorbed Mg^{28} was almost entirely evacuated within 36 hours this was not so with all our subjects and led to our accepting 96 hours as the end point. At this time in all our subjects residual excretion had fallen to a level compatible with re-excretion. In all the subsequent discussion the excretion at 96 hours will be taken as the end point.

In table 1 are seen the results obtained in 10 control subjects. The fecal excretion in 96 hours was 55.7 ± 13.4 per cent of the dose corresponding to an absorption of 9.3. ± 3.7 mEq. Mg/day. In seven of the subjects a total of 93 per cent of the Mg^{28} found in the stool was recovered in the first 48 hours. The subsequent fecal excretion was equal to 4.0 per cent (range 1.1 to 7.1 per cent/day) of the retained isotope per day. In three patients studied after intravenous injection of Mg^{28}, the fecal excretion reached only 0.45 to 1.08 per cent of the retained dose per day. The higher late excretion after oral dosage may be due to the intestinal mucosal cells acting as a reservoir of relatively high specific activity Mg^{28}.

The urinary excretion of 8.4 ± 3.5 per cent of the absorbed dose of Mg^{28} in 48 hours is lower than the 13.9 ± 5.7 per cent found after intravenous injection. This difference is to be expected because of the plasma specific activity per unit dose after intravenous dosage is higher than after oral administration.

Effects of Different Dietary Intake of Mg on Isotope Absorption

The effect of dietary change was tested in paired studies on three subjects. An example of fecal collection in one of these subjects is seen in figure 2. When the subject had been equilibrated on a dietary intake of 2.9 mEq./day only 21.4 per cent of the dose of isotope was excreted in the feces, i.e., 78.6 per cent of the dose was absorbed: on the other hand when the dietary intake was 52.7 mEq./day 27 per cent of the dose was absorbed.

The results for all three subjects were very similar. On the low dietary intake about three times as much isotope was absorbed as on the high intake. The absorption on our usual dietary intake fell approximately midway between these two extremes. On normal and high intakes the total magnesium absorption can be calculated for the isotope absorption. The results of table 2 show that increasing the dietary intake from 19.4 to 47.8 mEq./day, i.e., by a factor of 2.5 increased the magnesium absorption from 8.6 to 11.4 mEq./day, i.e., by only 33 per cent. The application of this approach to the low intake is not valid because the total dietary intake is of the same order as the amount of magnesium being resecreted so that considerable isotope dilution may occur in the intestine. The value shown in table 2 therefore underestimates the absorption. It will be noted that in all the subjects the serum magnesium was lower on the low intakes than when on the supplemented diets. This difference has been encountered in other studies and is probably genuine but owing to the small sample is not statistically significant ($p = 0.08$). It will also be seen that in the low diets the renal clearance was depressed and in the supplemented diet somewhat increased.

The change in absorption of Mg^{28} on the different dietary loads could be due either to the change in the total load of magnesium presented to the intestinal mucosa or to a regulatory function of the mucosa related to the state of the body reserves of magnesium. In the results just described the body state with regard to magnesium had been changed as demonstrated by a change in serum level and renal clearance. In two of the subjects the test was repeated with this difference: both subjects were equilibrated on the standard diet of 20 mEq./day for eight days. On the day of the test the isotope was administered with breakfast, but the breakfast corresponded in the case of F. W. to that of the high diet and in the case of M. C. to that of the low diet. During the subsequent period the subjects returned to the standard diet.

These results are shown in figure 3 where it can be seen that the effect of changing the magnesium content of the meal in which the isotope was administered was very similar to that found after equilibration on the equivalent diet. These results suggest then that the major reason for the difference between isotope absorption on varying dietary intakes was the acute magnesium load presented to the intestine and that adaptive changes in intestinal absorption did not occur.

Plasma Concentration of Mg^{28}

Figure 4 shows the course of the plasma radioactivity in three control subjects. It can be seen that appreciable amounts of radioactivity were present at

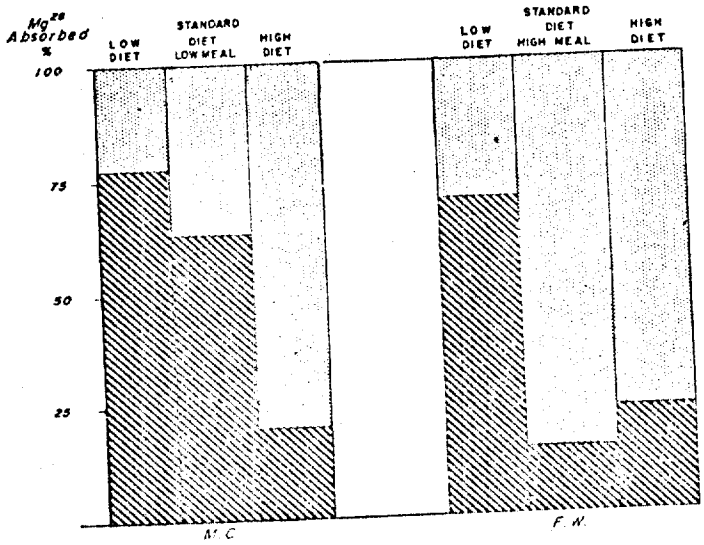


Fig. 3.—Per cent absorption of Mg^{28} in paired studies in two subjects. See text for details.

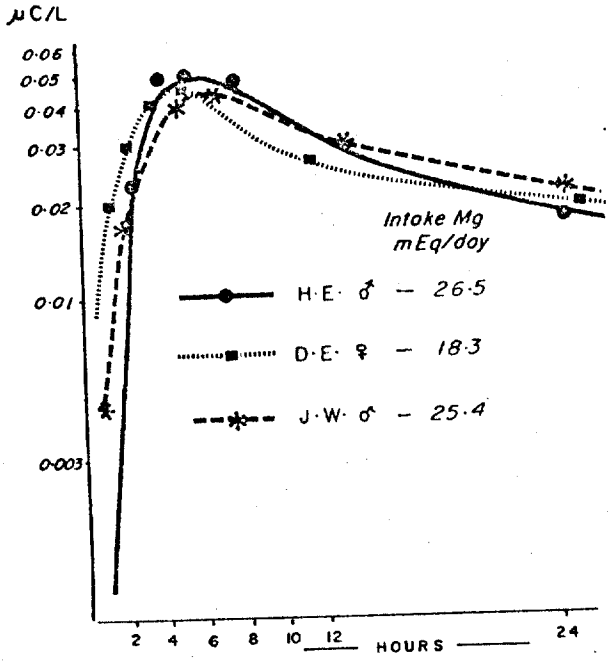


Fig. 4.—Plasma radioactivity in 3 control subjects following ingestion of 5 μc. Mg^{28}.

one hour and that the plasma concentration rapidly rose to a peak of 0.04 to 0.05 μc./L. at 5 to 6 hours and then slowly fell off so that at 24 hours the concentration was 0.018 to 0.022 μc./L. In the subjects on high and low dietary intakes the change in the percentage of isotope absorbed was reflected in

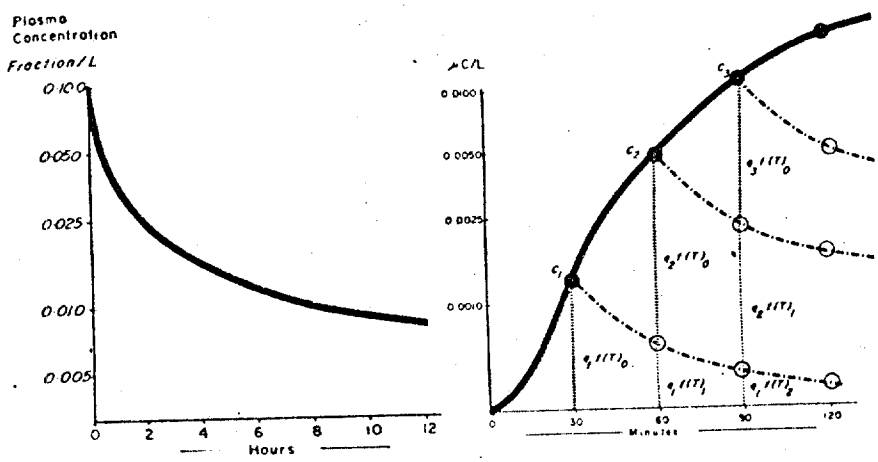


Fig. 6. *(left)*—Normal plasma disappearance curve following the injection of Mg^{28} intravenously. Mean results obtained in 15 normal subjects. Ordinate: fraction of retained dose per liter of plasma.

Fig. 7.—Method of calculating absorption from the plasma concentration. Solid line shows the actual plasma concentrations. The vertical lines show the analytic values. For example the calculation of C_3 according to the formula given on page 14 is

$$C_3 = q_1 f(T)_2 + q_2 f(T)_1 + q_3 f(T)_0.$$

The values found are $C_3 = 0.0110$ μc./L. $q_1 = 0.013$ μc., $q_2 = 0.042$ μc. $f(T)_2 = 0.036\ L^{-1}$, $f(T)_1 = 0.048\ L^{-1}$, $f(T)_0 = 0.100\ L^{-1}$ Solving for q_3: $0.0110 = (0.013 \times 0.036) + (0.042 \times 0.048) + (q_3 \times 0.100)$. Therefore, $q = 0.085$ μc.; i.e. 0.085 μc. is absorbed in the 30 minute period from q_2 (1 hour) to q_3 (1.5 hours).

relates amount of radioactivity injected (q) to the concentration in the plasma (c). This parameter may be expressed symbolically as $f(T)_n$ where the subscript defines the point in time. Thus

$$c_n = q\, f(T)_n$$

With intestinal absorption the plasma concentration at any time n is

$$c = q_1 f(T)_{n-1} + q_2 f(T)_{n-2} + q_3 f(T)_{n-3} + \ldots + q_{n-1} f(T)_1 + q_n f(T)_0.$$

Here q_1 is the amount of Mg^{28} absorbed in the interval t = o to t = 1; q_2 is the amount in the interval t = 1 to t = 2, etc. The total amount absorbed at any time (Q_n) is the sum of the fractional absorptions, thus

$$Q_n = q_1 + q_2 + q_3 \ldots . q_{n-1} + q_n.$$

Extrapolation of Q_n to infinity should give the total amount of isotope absorbed and if the analytic procedure is valid this should agree with the absorption as determined by direct stool determinations.

Figure 7 illustrates the graphical reconstruction of the plasma curve. Figure 8 illustrates the results obtained with this method in the subject (J. W.) whose fecal excretion curve is shown in figure 1. It can be seen that absorption began within an hour of the start of the meal and reached a steady level of about 5.5 per cent of the dose per hour after 2 to 2½ hours which was

the plasma curves. In figure 5 the plasma curves from one subject (M. C.) are seen. On the low diet when absorption was high the peak plasma radioactivity was 0.098 μc./L. whereas on the high diet when absorption was only a third as great the peak radioactivity was only 0.033 μc./L. Similar results were found in the other two subjects (table 3). The time to maximum plasma concentration was not affected by the difference in diet.

The reproducibility of these plasma curves and the relatively early time at which the peak concentration was reached suggested that analytic treatment might give useful information about the course of intestinal absorption of magnesium.

Since absorption occurs into the portal circulation, for plasma analysis to be adequate there must not be appreciable trapping of isotope in the liver. There is no satisfactory information about this although it seems unlikely, so that strictly speaking our analysis relates to Mg^{28} that has been absorbed, passed the liver, and been delivered into the systemic circulation through the hepatic veins. Presumably magnesium entering the circulation through these veins suffers the same fate as magnesium injected into an arm vein.

To facilitate calculation the analysis used is discontinuous and based on the assumption that the gradual rise in plasma concentration over a relatively short period from t_0 to t_1 can be equated to the sudden rise of concentration produced by the intravenous injection of radioactivity at time t_1. The disappearance of this activity from the circulation is then calculated for the mean plasma disappearance curve obtained on a group of normal subjects. Since the period of integration used in the calculation is 0.5 hour the early very rapid phase of disappearance of Mg^{28} from the circulation is not relevant. The standard curve used for this purpose is seen in figure 6. This curve also

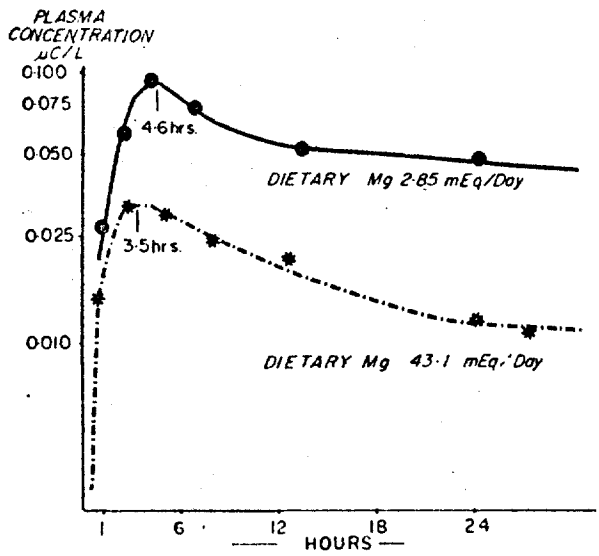


Fig. 5.—Plasma radioactivity in a single subject on low ●——● and high (★——★) dietary intake of magnesium following administration of 5 μc. of Mg^{28}.

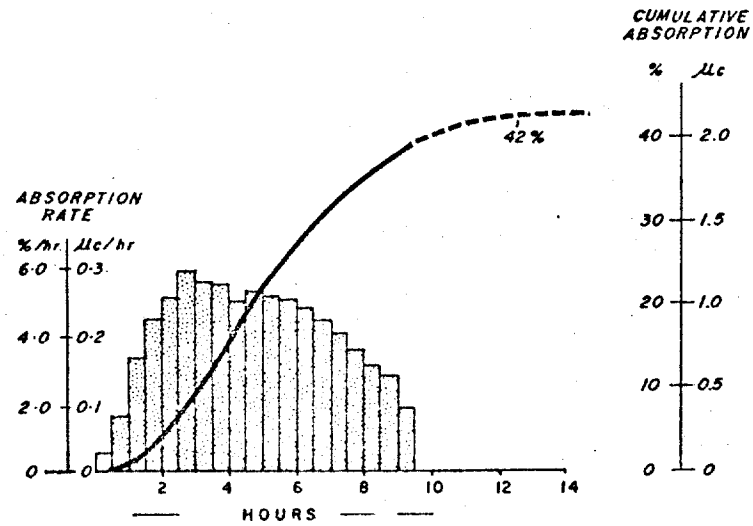


Fig. 8.—Rate of absorption following oral administration of 5 μc. Mg^{28}. Hatched columns represent rates of absorption calculated from the plasma radioactivity. ——, cumulative absorption of Mg^{28}.

maintained for a further 4 to 5 hours, and then gradually fell off. The absorption was half complete in 4.8 hours. The integral curve showed that a total of 42 per cent of the dose was absorbed. This was identical with the value obtained by fecal analysis. The agreement in 7 of the 8 other cases was equally good and showed no evidence of any systematic error in the procedure (fig. 9). In the one case where agreement was poor (H. E., fecal measurement 65 per cent absorption; plasma analysis, 47 per cent) it is difficult to know which method was in error. Table 3 summarizes the data obtained with this method on standard, low and high dietary intakes. This method is similar to that used by Scholer and Code[21] for the study of the absorption of water.

It can be seen that the course of absorption is not dependent on the dietary load. At the time when the plasma level was at its peak about 77 per cent of the total absorbed Mg^{28} had already been absorbed. The maximum percentage of the administered dose absorbed per hour is of course related to the dietary intake ranging from 3.8 per cent/hr. on the high diet to 10.7 per cent/hr. on the low diet. On the other hand it is interesting that a constant percentage of the total amount absorbed was absorbed per hour.

Discussion

The preceding show that after ingestion of radioactive magnesium in a meal absorption can often be detected within an hour. This may be partly due to absorption through the gastric mucosa (Kasel[19]), but probably represents also early passage into the duodenum. In all subjects the rate of absorption had reached a steady level at 2 to 3 hours and continued more or less constant for the next 4 to 6 hours by which time approximately 80 per cent of the total absorption had occurred. This period corresponds fairly

Table 3.—*Rate of Absorption of Mg^{28}*

Subject	diet	Maximum plasma Mg^{28} (μc./L.)	Time for maximum plasma conc. (hr.)	Time for 50% absorption (hr.)	% Mg^{28} Absorbed from: Plasma analysis	% Mg^{28} Absorbed from: Fecal determination	Maximum Absorption Rate: % dose /hr.	Maximum Absorption Rate: % Amount absorbed /hr.
H. E.	Standard	0.050	5.5	5.0	47	65	9.6	14.7
J. W.	"	0.047	7.5	4.8	42	42	5.4	12.8
D. E.	"	0.046	5.0	3.5	36	33	6.0	16.7
		0.048	6.0	4.4	42	47	7.0	14.6
F. W.	Low	0.079	6.3	3.9	82	71	10.6	12.8
D. J.	"	0.058	5.0	4.5	64	78	8.2	12.8
M. C.	"	0.098	4.3	3.7	75	78	13.4	17.8
		0.078	5.2	4.0	74	76	10.7	14.5
F. W.	High	0.029	6.5	3.7	25	23	3.0	11.9
D. J.	"	0.031	4.2	3.2	26	27	4.2	16.4
M. C.	"	0.033	3.7	3.0	24	21	4.2	17.5
		0.031	4.8	3.3	25	24	3.8	15.3

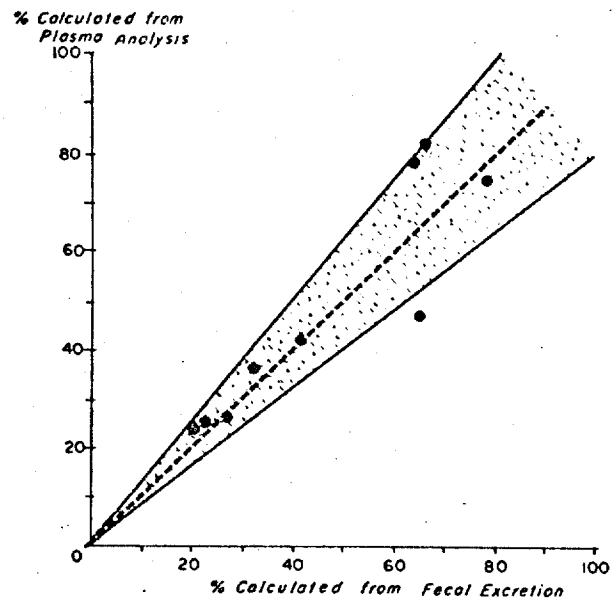


Fig. 9.—Correlation of Mg^{28} absorption as measured by fecal collection and analysis of plasma radioactivity. The shaded area includes ±20 per cent deviation.

well with the time taken for a meal to traverse the small intestine and suggests more or less uniform absorption occurs along the length of the small intestine. In any case absorption clearly occurs in the upper as well as the lower small intestine. Schacter et al.[14] have recently found in an *in vitro* study of absorption of calcium[45] that the most active absorption occurs in the duodenum and upper small intestine in contrast to the commonly accepted view that absorption is mainly a function of the ileum. Aikawa[13]

has shown that Mg^{28} is not absorbed from the rabbit large bowel. Our results also suggest this in that little if any further absorption is occurring in the 12 to 36 hour period when much of the unabsorbed isotope is lying in the large bowel. The over-all absorption of Mg^{28} on the 20 mEq. diet agrees fairly well with the results of Aikawa et al.[10] and means that as judged by our procedure a little less than half of the dietary intake of magnesium is normally absorbed. Like most isotope studies of absorption our results are open to the objection that not all the dietary magnesium may be equally absorbable and that radioactive exchange between the added Mg^{28} was very probably incomplete. In the patients who were in a steady state with respect to total body magnesium the urinary output of magnesium was only slightly lower than the amount calculated to be absorbed suggesting that any error from this source is not great.

Secretion of injected Mg^{28} into the gut amounted to about 0.8 per cent of the body load per day from which it can be calculated that approximately 2 mEq. of total magnesium are secreted per day. This represents a considerable total compared with the 8 to 9 mEq./day absorbed in the standard diet. While this resecretion represents a relatively trivial amount of Mg^{28} being resecreted it does represent a significant amount of isotope dilution for the magnesium in the intestine. In effect when we talk of absorption from 20 mEq. diet, the total pool is about 22 mEq., and in the case of the 2.8 mEq. diet the pool is about 4.8 mEq. Bearing this in mind the rate of Mg^{28} absorption does not seem to be linearly related to load although it is certainly influenced by load. Our results do not support the possibility that intestinal absorption of magnesium is affected to any important extent by depletion or repletion of body magnesium, not at least when this has been carried out over as short a period as five days and is effectively evidenced by changes in serum magnesium and urinary clearance. It is very difficult to produce any serious depletion of magnesium experimentally in man (Fitzgerald and Fourman),[6] and it may be that if a severe depletion could be produced comparable to those in animals (MacIntyre and Davidsson[20]), the result might be quite different. Since magnesium absorption appears to occur more or less uniformly along the intestine it might be expected that defective absorption in intestinal resection would be more or less proportional to the percentage of the small intestine resected.

It has been reported that absorption of magnesium from the intestine is greater when the associated anion is acetate than when it is sulphate. It seems most unlikely that such an effect could depend on the sulphate ion per se, but is more likely to be due to the poorer absorbability of the sulphate than acetate ion with greater fluid retention in the gut and more rapid transit. Since the rate of absorption of magnesium is slow more rapid transit might be expected to lead to decreased absorption. It is also likely that drugs changing the motility of the small intestine would also lead to changes in magnesium absorption.

REFERENCES

1. Duckworth, J., and Warnock, G. M.: Magnesium requirements of man in relation to calcium requirements, with observations on adequacy of diets in common use. Nutrition Abstr.& Rev. 12:167, 1942.

2. Flink, E. B.: Magnesium deficiency in man. J.A.M.A. *160*:1406, 1956.
3. Elkinton, J. R.: The role of magnesium in the body fluids. Clin.Chem. (Supp.) *3*:319, 1957.
4. Sherman, H. C.: Cemistry of Food and Nutrition. New York, The MacMillan Company, 1957, 8th ed.
5. Tibbetts, D. M., and Aub, J. C.: Magnesium metabolism in health and disease. I. The magnesium and calcium excretion of normal individuals, also the effects of magnesium, chloride and phosphate ions. J.Clin.Invest. *16*: 491, 1937.
6. Fitzgerald, M. G., and Fourman, P.: An experimental study of magnesium deficiency in man. Clin.Sc. *15*:635-647, 1956.
7. Barnes, B. A., Cope, O., and Harrison, T.: Magnesium conservation in the human being on a low magnesium diet. J.Clin.Invest. *37*:431, 1958.
8. Zumoff, B., Bernstein, E. H., Imarisio, J. J., and Hallman, L.: Radioactive magnesium (Mg^{28}) metabolism in man. Clin.Res. *6*:260, 1958.
9. Caesar, J. J., Graham, L. A., and Burgen, A. S. V.: To be published.
10. Aikawa, J. K., Rhoades, E. L., and Gordon, G. S.: Urinary and fecal excretion of orally administered Mg^{28}. Proc.Soc.Exper.Biol.& Med. *98*:29, 1958.
11. Shohl, A. T.: Mineral Metabolism. American Chemical Society Monograph. New York, Rheinhold Publishing Company, 1939, no. 82.
12. Dempsey, E. F., Carroll, E. L., Albright, F., and Hanneman, P. H.: A study of factors determining fecal electrolyte excretion. Metabolism *7*:108, 1958.
13. Aikawa, J. K.: Gastrointestinal absorption of Mg^{28} in rabbits. Proc.Soc. Exper.Biol.& Med. *100*:293, 1959.
14. Schacter, D., and Rosen, S. M.: Active transport of Ca^{45} by the small intestine and its dependence on vitamin D. Am.J.Physiol. *196*:357, 1959.
15. McCance, R. A., and Widdowson, E. M.: The Chemical Composition of Foods. London, Medical Research Council Special Report Series, 1946, no. 235, 2nd ed.
16. Barnes, B. A., Eddison, G. G., and Cope, O.: Preparation and bio-assay of a low magnesium liquid diet for human consumption. Surgery *42*:643, 1957.
17. Table of Food Values Recommended for Use in Canada. Ottawa, Nutrition Division, Department of National Health and Welfare. 1958, 2nd ed.
18. Andreasen, E.: On the determination of magnesium in serum and urine by the Titan Yellow method. Scandinav. J.Clin.& Lab.Invest. *9*:138, 1957.
19. Kasel, L.: Gastric absorption. Physiol. Rev. *28*:433, 1948.
20. MacIntyre, I., and Davidsson, D.: The production of secondary potassium depletion, sodium retention, nephrocalcinosis and hyperglycemia by magnesium deficiency. Biochem.J. *70*:456, 1958.
21. Scholer, J. F., and Code, C. F.: Rate of absorption of water from stomach and small bowel of human beings. Gastroenterology *27*:565, 1954.

The benzene chain contained in both drugs is possibly responsible for the severe reaction and the disappearance of the granulocytes from the blood.

A number of similar cases have been reported recently.

A difference may be expected in the reaction to benzene quickly absorbed from the alimentary tract, as compared to the type of benzene poisoning that may result from the prolonged, slow absorption that occurs in industrial exposure.

Individuals are known to vary greatly in their susceptibility to benzene. Granulopenia following the use of barbiturates and amidopyrine may therefore occur only in those individuals who are unusually susceptible to the benzene chain.

Forty-Fourth and Mill Creek Parkway.

CLINICAL MANIFESTATIONS OF HIGH AND LOW PLASMA MAGNESIUM

DANGERS OF EPSOM SALT PURGATION IN NEPHRITIS

ARTHUR D. HIRSCHFELDER, M.D.
WITH THE TECHNICAL ASSISTANCE OF
VICTOR G. HAURY, B.A.
MINNEAPOLIS

Although a great deal of clinical significance has been attached to variations in blood calcium, variations in blood magnesium have entirely escaped notice. Very little has been known even about what happens when a patient takes an ordinary purgative dose of epsom salt. Matthew Hay[1] found that one normal man excreted 28 per cent of the ingested magnesium through the kidneys in twenty-four hours, and Yvon[2] found 21 per cent. Using a new and convenient method for the quantitative determination of magnesium in blood plasma and urine, we[3] have found that seven normal men excreted from 40 to 44 per cent (average 42.6 per cent) of the magnesium taken in a single ordinary purgative dose of epsom salt within twenty-four hours after ingestion. However, in spite of the large amount of magnesium absorbed, the concentration of magnesium in the blood plasma (normal, from 1.8 to 2.5 mg. of magnesium per hundred cubic centimeters; average normal, 2.09) is scarcely affected (average increase, 0.24 mg. of magnesium per hundred cubic centimeters; maximum increase, 0.41 mg.). In normal dogs and normal rabbits the percentage of ingested magnesium that is excreted and the concentration of the magnesium in the blood plasma are almost exactly the same as in man, and with corresponding doses by mouth the plasma concentration also remains practically unchanged. In animals the percentage of the ingested magnesium that is excreted in the urine is practically constant, regardless of the dose ingested or of the concentration of magnesium in the blood plasma.

HIGH PLASMA MAGNESIUM (HYPERMAGNESEMIA) AND EPSOM SALT PURGATION

However, when the kidneys are injured, the results are totally different. We have found in animals in which renal injury had been produced experimentally that less magnesium is excreted than normally. The excretion of magnesium is less in animals in which the tubules are more severely injured than the glomeruli (corrosive mercuric chloride nephrosis) than in those in which the glomeruli are injured more than the tubules (cantharides glomerulonephritis). The decrease in excretion of magnesium corresponds quite closely to the decrease in excretion of phenolsulphonphthalein but does not correspond to the alterations in excretion of xylose. We have found also that in nephrectomized animals and in animals with renal, and especially tubular, insufficiency the ingestion of magnesium sulphate causes a tremendous and rapid rise in plasma magnesium, sufficient even to bring them into a state of coma. Coma occurred whenever the plasma calcium reached or exceeded 17 mg. of magnesium per hundred cubic centimeters of plasma. This exactly coincides with the level at which Neuwirth and Wallace[4] and Taylor and Winter[5] found coma setting in after the subcutaneous injection of magnesium salts.

We have therefore studied the effects of the ingestion of ordinary purgative doses of magnesium sulphate on the plasma magnesium and the general physical condition in a series of patients suffering from renal disease. In all these patients, just as in the nephritic animals, a tremendous rise in plasma magnesium occurred within from four to six hours after an ordinary purgative dose of magnesium sulphate (epsom salt) had been taken by mouth. While one such dose was not sufficient to raise the magnesium concentration in the plasma to the level at which coma sets in (about 17 mg. of magnesium per hundred cubic centimeters of plasma), it often did rise to about two thirds of that concentration (from 9 to 11 mg.) and a number of the patients did show a decidedly increased drowsiness or even a light coma accompanying the increase in plasma magnesium. The results are indicated in chart 1.

NOTES ON CASES

Normal.—Plasma:[6] nonprotein nitrogen, 30 mg.; creatinine, from 1.5 to 2.0 mg.; magnesium, from 1.85 to 2.09 mg. After taking from 20 to 30 Gm. of epsom salt ($MgSO_4.7H_2O$): average increase, 0.24 mg.; maximum increase, 0.4 mg. Excreted in urine: phenolsulphonphthalein, from 60 to 80 per cent in two hours; magnesium excreted in twenty-four hours, from 40 to 44 per cent (average 42.6 per cent) of the magnesium ingested.

HIGH PLASMA MAGNESIUM

CASE 1.—*Subacute glomerulonephritis.* W. A., a woman, aged 41, in the Minneapolis General Hospital, complained of edema. Plasma: nonprotein nitrogen, 88; creatinine, 5.5; magnesium, 2.98; calcium, 11.3. Urine: phenolsulphonphthalein, 39 per cent. After 25 Gm. of epsom salt by mouth: Plasma: magnesium, 5.5; calcium, 13.0. Magnesium excreted in twenty-four hours: 23.8 per cent. Little effect on mental activity.

From the Department of Pharmacology, University of Minnesota.

This paper was announced for presentation before the Section on Pharmacology and Therapeutics of the American Medical Association at the Milwaukee Session, June 15, 1933.

This investigation was made possible by a grant of funds from the Therapeutic Research Committee of the American Medical Association. Most of the patients on whom the observations were made were in the medical service of Dr. Hobart A. Reimann at the University of Minnesota Hospital and of Dr. George E. Fahr at the Minneapolis General Hospital. One was from the private practice of Dr. Charles B. Wright and three from the neurologic service of Dr. Joseph C. Michael at the Minneapolis General Hospital, all of whose cooperation is deeply appreciated. Preliminary reports have been published in Proceedings of the American Society for Clinical Investigation (J. Clin. Investigation 11: 841 (July) 1932, 12: 918, 1933), Proceedings of the American Society for Pharmacology and Experimental Therapeutics (J. Pharmacol. & Exper. Therap. 48: 277, 1933) and Proceedings of the Society for Experimental Biology and Medicine (Proc. Soc. Exper. Biol. & Med. 30: 995, 1933).

1. Hay, Matthew: J. Anat. & Physiol. 17: 222, 1883.
2. Yvon, P.: Arch. de physiol. norm. et path. 5: 304, 1898.
3. Hirschfelder, A. D., and Serles, E. R.: J. Clin. Investigation 11: 841 (July) 1932; J. Biol. Chem., March, 1934.
4. Neuwirth, L., and Wallace, G. B.: J. Pharmacol. & Exper. Therap. 35: 171 (Feb.) 1929.
5. Taylor, W. F., and Winter, J. E.: J. Pharmacol. & Exper. Therap. 35: 435 (April) 1929.

6. Amounts in plasma are always milligrams per hundred cubic centimeters.

CASE 2.—*Nephrosis.* L., a woman, aged 25, in the Minneapolis General Hospital, was mentally alert. Plasma: nonprotein nitrogen, 73; creatinine, 4.7; magnesium, 2.98; calcium, 11.3. Urine: phenolsulphonphthalein, 29 per cent. Mentally active. After 20 Gm. of epsom salt by mouth: Plasma: magnesium, 7.75; calcium, 14.9. Urine: magnesium excreted, 21.7 per cent. Listless and drowsy.

CASE 3.—*Acute glomerulonephritis.* M. R., a man, aged 33, in the University Hospital, with a blood pressure of 150 systolic, 100 diastolic, was mentally alert. Plasma: nonprotein nitrogen, 60; creatinine, 4.9; magnesium, 1.87; calcium, 9.8. Urine: albumin + +; phenolsulphonphthalein, 39 per cent. After 30 Gm. of epsom salt by mouth: Plasma: magnesium, 7.9; calcium, 14.8. Urine: magnesium excreted, 26.3 per cent. Near coma, vomiting.

CASE 4.—*Acute glomerulonephritis.* K., a woman, aged 57, in the Minneapolis General Hospital, with a blood pressure of 170 systolic, 115 diastolic, was mentally active. Plasma: nonprotein nitrogen 98; creatinine, 8.9; magnesium, 2.73; calcium, 11.9. Urine: albumin + + +; phenolsulphonphthalein, 59 per cent. After 20 Gm. of epsom salt by mouth: Plasma: magnesium, 9.22; calcium, 15.7. Urine: magnesium, 20.7 per cent. Very drowsy, near coma.

CASE 5.—*Chronic glomerulonephritis.* L. J., a woman, aged 35, in the University Hospital, was inactive and drowsy. Plasma: nonprotein nitrogen, 136; creatinine, 12; magnesium, 3.9; calcium, 19.9. Urine: albumin + + +; phenolsulphonphthalein, 26 per cent. After 25 Gm. of epsom salt by mouth: Plasma: nonprotein nitrogen, 136; creatinine, 12; magnesium, 16.5 per cent. Light coma.

CASE 6.—*Chronic glomerulonephritis.* R. A., a man, aged 60, in the Minneapolis General Hospital, with a blood pressure of 190 systolic, 125 diastolic, was mentally alert. Plasma: nonprotein nitrogen, 185; creatinine, 17.5; magnesium, 4.78; calcium, 15.2. Urine: albumin + + + +; phenolsulphonphthalein, 15 per cent. After 25 Gm. of epsom salt: Plasma: magnesium, 10.35; calcium, 23.5. Urine: magnesium, 10 per cent. Light coma, hard to awaken.

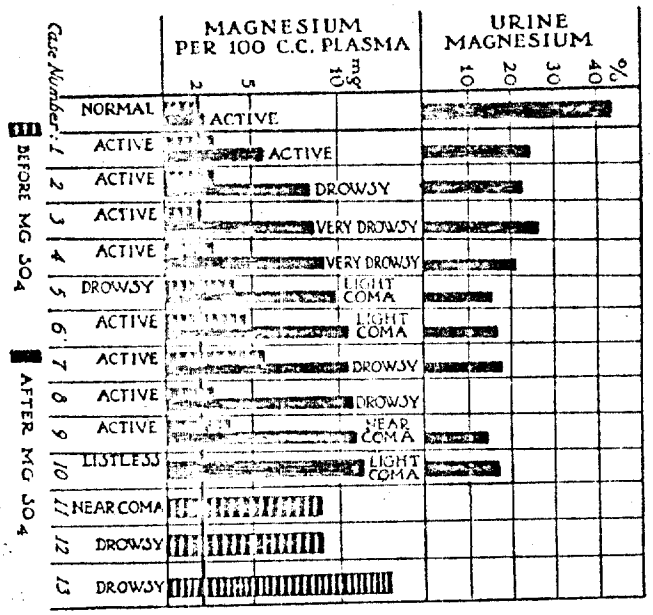


Chart 1.—Observations in cases showing high plasma magnesium: Urine magnesium = percentage of ingested magnesium excreted in urine in twenty-four hours. Before magnesium sulphate = milligrams of magnesium per hundred cubic centimeters of plasma before taking epsom salt ($MgSO_4$, $7H_2O$) by mouth. After magnesium = milligrams of magnesium per hundred cubic centimeters of plasma from four to six hours after taking epsom salt by mouth. Words below the cross-hatched lines refer to mental condition before taking epsom salt; those above the solid line represent mental condition from four to six hours after taking epsom salt.

CASE 7.—*Acute glomerulonephritis.* E. P., a man, aged 43, in the Minneapolis General Hospital, with a blood pressure of 165 systolic, 110 diastolic, was mentally alert. Plasma: nonprotein nitrogen, 85; creatinine, 8.75; magnesium, 5.75; calcium, 19.75. Urine: albumin + + +; phenolsulphonphthalein, 15 per cent. After 30 Gm. of epsom salt by mouth: Plasma: magnesium, 10.5; calcium, 24.1. Urine: magnesium, 18.5 per cent. Very drowsy.

CASE 8.—*Acute glomerulonephritis.* E. R., a woman, aged 39, in the University Hospital, with a blood pressure of 150 systolic, 110 diastolic, was very alert. Plasma: nonprotein nitrogen, 100;

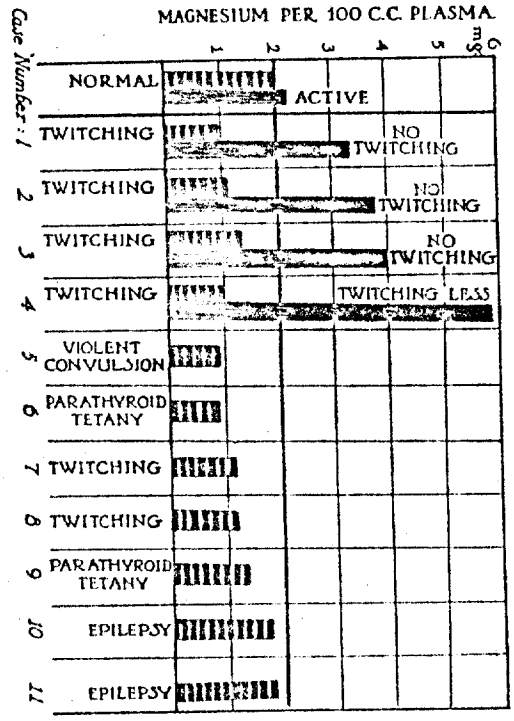


Chart 2.—Observations in cases showing low plasma magnesium. Legends have same significance as in chart 1.

creatinine, 9; magnesium, 2.49; calcium, 11.0. Urine: albumin + + +; phenolsulphonphthalein, 27.5 per cent. After 30 Gm. of epsom salt: Plasma: magnesium, 10.75; calcium, 17.8. Drowsy.

CASE 9.—*Chronic glomerulonephritis.* E. L., a woman, aged 27, in the University Hospital, with a blood pressure of 200 systolic, 135 diastolic, was alert. Plasma: nonprotein nitrogen, 265; creatinine, 23; magnesium, 4.1; calcium, 16.9. Urine: albumin + + + +; phenolsulphonphthalein, 12 per cent. After 20 Gm. of epsom salt by mouth: Plasma: magnesium, 10.91; calcium, 18.5. Urine: magnesium, 15.35. Very drowsy, difficult to awaken.

CASE 10.—*Acute glomerulonephritis.* O. L., a man, aged 58, in the University Hospital, with a blood pressure of 185 systolic, 140 diastolic, was listless. Plasma: nonprotein nitrogen, 195.5; creatinine, 25.3; magnesium, 4.25; calcium, 19.5. Urine: albumin + + +; phenolsulphonphthalein, 12 per cent. After 30 Gm. of epsom salt by mouth: Plasma: magnesium, 11.32; calcium, 25.9. Urine: magnesium, 17.5 per cent. Hard to awaken, light coma.

CASE 11.—*Chronic glomerulonephritis.* J. J., a woman, aged 62, in the University Hospital, with a blood pressure of 175 systolic, 125 diastolic, near coma. Plasma: nonprotein nitrogen, 215; creatinine, 25; magnesium, 8.93; calcium, 19.7. Urine: albumin + + +; phenolsulphonphthalein, 12.8. No epsom salt given.

CASE 12.—*Subacute glomerulonephritis.* O. J., a woman, aged 31, in the University Hospital, was very dull and listless. Plasma: nonprotein nitrogen, 190; creatinine, 22; magnesium, 9.0; calcium, 24.0. Urine: albumin + + + +; phenolsulphonphthalein, 17 per cent.

CASE 13.—*Acute glomerulonephritis.* F. S., a man, aged 52, in the University Hospital, with a blood pressure of 190 systolic, 115 diastolic, was very drowsy. Plasma: nonprotein nitrogen, 190; creatinine, 21.5; magnesium, 13.0; calcium, 22.0. Urine: albumin + + + +; phenolsulphonphthalein, 5 per cent; total urine, 150 cc. Died. Autopsy showed arteriosclerotic kidneys.

Since a single ordinary dose of epsom salt by mouth can raise the concentration of magnesium in the blood of nephritic patients to two thirds of the concentration at which coma sets in and since with larger doses it is easy to induce coma in nephritic animals, it seems probable that a few repeated doses of epsom salt would induce coma in the patients. It is therefore most probable that there are every year in the United States many hundreds of cases of coma occurring in nephritic patients which are diagnosed uremic coma but which in reality are magnesium coma caused by the use of epsom salt as a purgative. Since experimental animals could be brought out of this coma instantly and their lives could be prolonged by the intravenous injection of calcium chloride, the intravenous injection of calcium chloride would probably restore such patients to consciousness.

We are not stating, nor do we think, that all or even most cases of uremic coma are due to magnesium intoxication, but we are merely calling attention to the fact that a condition of coma easily mistaken for uremic coma can be induced in nephritic patients by epsom salt purgation. Magnesium citrate would unquestionably have the same effect.

Our results in no way contradict those of Blackfan and his collaborators,[7] who have administered magnesium sulphate intravenously for the control of uremic convulsions, or those of Dorsett[8] and McNeile and Vruwink[9] for eclamptic convulsions. Since they gave intravenously in carefully controlled doses only sufficient magnesium sulphate to produce subsidence of the convulsions, they stopped the administration as soon as a sufficiently high level of magnesium concentration in the blood had been reached. However, when the drug is administered by mouth and absorbed continuously from the intestine without regard to its concentration in the blood, this level can easily be exceeded.

Since our experiments on animals with renal insufficiency have shown that coma cannot be induced by tremendous doses of sodium sulphate, it would appear that sodium sulphate should be the saline purgative of choice in patients with renal disease.

Our experiments on animals[10] showed also that animals whose plasma magnesium was only slightly raised (to 5 mg. or more) were much more sensitive than normal animals to ordinary doses of morphine.[7] This explains Dr. Osler's old aphorism that "in severe nephritics and very old persons morphine should be given with caution." Epsom salt was Dr. Osler's favorite saline purgative and was administered almost as a routine in his wards at Johns Hopkins Hospital.

LOW PLASMA MAGNESIUM (HYPOMAGNESEMIA)

Since Kruse, Orent and McCollum[11] have induced a form of tetany in rats that were raised on a diet free from magnesium, it seemed probable that cases showing convulsions or other conditions of increased neuromuscular activity would be encountered clinically in which the plasma magnesium is low.

We have encountered thus far ten such clinical cases, in each of which convulsions or muscular twitchings were present. The data of these cases are shown in chart 2.

CASE 14.—*Chronic glomerulonephritis, hypertension and convulsions.* S., a woman, aged 53, at the University Hospital, complained of edema of the legs and arms, and of intermittent general muscular twitchings. Plasma: nonprotein nitrogen, 200; creatinine, 15; magnesium, 0.95; calcium, 10.8. Urine: albumin + + +; phenolsulphonphthalein, 12 per cent. After 20 Gm. of epsom salt by mouth: Plasma: magnesium, 3.3; calcium 11.9. No more twitching.

CASE 15.—*Chronic glomerulonephritis.* C. O., a man, aged 36, in the Swedish Hospital, had general muscular twitching. Plasma: nonprotein nitrogen, 76.5; creatinine, 5.5; magnesium, 1.09; calcium, 9.3. Urine: albumin + +; phenolsulphonphthalein, 22 per cent. After 20 Gm. of epsom salt by mouth: Plasma: magnesium, 3.78; calcium, 13.0. No twitching.

CASE 16.—*Chronic glomerulonephritis.* P. S., a man, aged 51, at the Minneapolis General Hospital, was nervous, and had general muscular twitching. Plasma: nonprotein nitrogen, 55.0; creatinine, 4.3; magnesium, 1.31; calcium, 16.7. Urine: albumin + +; phenolsulphonphthalein, 33 per cent. After 25 Gm. of epsom salt by mouth: Plasma: magnesium, 4.0; calcium, 17.3. No twitching; resting quietly.

CASE 17.—*Eclampsia.* I. H., a woman, aged 47, at the University Hospital, was pregnant and had frequent headaches and general muscular twitchings passing into convulsions. Plasma: Nonprotein nitrogen, 33; creatinine, 2.1; magnesium, 1.0; calcium, 11.3. After 25 Gm. of magnesium sulphate by mouth: Plasma: magnesium, 5.87; calcium, 14.13. No twitching or convulsions.

CASE 18.—*Glomerulonephritis.* W. C., a man, aged 51, at the Northwestern Hospital, had violent convulsions (uremia) and a blood pressure of 160 systolic, 100 diastolic. Plasma: nonprotein nitrogen, 175; creatinine, 18; magnesium, 0.9; calcium, 24.0. No epsom salt was given.

CASE 19.—*Parathyroid deficiency.* H., a man, aged 61, in the University Hospital, with a blood pressure of 130 systolic, 90 diastolic, had marked convulsions. Plasma: nonprotein nitrogen, 30; creatinine, 1.8; magnesium, 0.93; calcium, 7.0. No epsom salt was given.

CASE 20.—*Possible cerebral injury.* M., a woman, aged 20, in the University Hospital, had general muscular twitching. The physical examination was negative and an encephalogram was negative. Plasma: magnesium, 1.14; calcium, 10.0. No epsom salt was given.

CASE 21.—*Epilepsy (?).* McK., a boy, aged 12 years, in the University Hospital, had intermittent twitching of the upper and lower extremities every two or three days, followed by sleep for several hours (possible epilepsy). Plasma: magnesium, 1.16; calcium, 11.8. No epsom salt was given. Was relieved later by ketogenic diet.

CASE 22.—*Parathyroid deficiency.* B., a woman, aged 42, in the University Hospital, had light convulsion. The laboratory report was negative. Plasma: magnesium, 1.35; calcium, 9.0. No epsom salt was given.

CASE 23.—*Epilepsy.* E. E., a woman, aged 35, in the Minneapolis General Hospital, had epileptic attacks every two to three months and was restless between attacks. The laboratory report was negative. Plasma: magnesium, 1.77; calcium, 12.9. No epsom salt was given.

CASE 24.—*Epilepsy.* D., a woman, aged 42, had epilepsy (grand mal, occasional attacks). The laboratory report was negative. Plasma: magnesium, 1.8; calcium, 13.7. No epsom salt was given.

It will be noted that two of these are cases of parathyroid tetany. This accords with the experimental results of McCollum and his collaborators and with the clinical observations of Howland and Marriott,[12] Denis

7. Blackfan, K. D., and Hamilton, Beget: Boston M. & S. J. **193:** 617 (Oct.) 1925. Blackfan, K. D., and McKhann, C. F.: Acute Glomerular Nephritis in Children, J. A. M. A. **97:** 1052 (Oct. 10) 1931.

8. Dorsett, L.: Am. J. Obst. & Gynec. **11:** 227 (Feb.) 1926.

9. McNeile, L. G., and Vruwink, John: Magnesium Sulphate Intravenously, J. A. M. A. **87:** 2[illegible] (July 24) 1926.

10. Hirschfelder, A. D., and Haury, V. G.: Proc. Soc. Exper. Biol. & Med. **20:** 1157 (May) 1933.

11. Kruse, H. D.; Orent, E. R., and McCollum, E. V.: J. Biol. Chem. **96:** 519 (May) 1932, **100:** 6[illegible] (May) 1933. Orent, E. R.; Kruse, H. D., and McCollum, E. V.: Am. J. Physiol. **101:** 454 (Aug.) 1932. Kruse, H. D.; Schmidt, Marguerite M., and McCollum, E. V., ibid. **105:** 645 (Sept.) 1933.

12. Howland, John, and Marriott, W. McK.: Quart. J. Exper. Med. **11:** 289 (July) 1918.

and Talbot,[13] and Bulger and Gausmann,[14] who also found low blood magnesium in some but not in all cases of parathyroid tetany. Moreover, Salvesen and Linder[15] and Lang[16] found that removal of the parathyroid glands in animals caused no change in blood magnesium.

Three of our patients with low plasma magnesium suffered from idiopathic epilepsy. Denis and Talbot also had reported two. But since we have also found practically normal plasma magnesium in four epileptic patients (1.77, 1.97, 2.13 and 2.6 mg., respectively) it is evident that a low plasma magnesium is not a constant finding in this disease. It is possible, however, that there may be a variability in the plasma magnesium of such individuals and that periods of low magnesium may precede and accompany the attacks. We are at present engaged in a further study of this question.

Four of the patients with low plasma magnesium had glomerulonephritis. That this should occur in spite of the diminished ability of the nephritic kidney to excrete magnesium may seem paradoxical, but since such patients are usually on a milk or at least on a soft diet, it is evident that their intake of magnesium salts can easily remain below their power to excrete magnesium. When the usual purgative dose of epsom salt was administered by mouth to three of these patients, the plasma magnesium rose within from four to six hours from the previous concentrations of 0.95, 1.09 and 1.37 mg. per hundred cubic centimeters, respectively, to 3.3, 3.78 and 4.0 mg., nearly double the normal concentration; and simultaneously with this increase in magnesium the twitchings or convulsions subsided.

Denis and Talbot have reported also one case of lobar pneumonia and one case of bronchopneumonia, both of which presented convulsions, and one case of chorea with low blood magnesium. However, we have had a case of chorea with normal plasma magnesium (2.13 mg.) and normal calcium (12.2 mg.).

In view of the cases here reported, it is evident that there is a clinical syndrome associated with low plasma magnesium accompanied by a condition of hyperirritability of the neuromuscular system, often associated with muscular twitchings or convulsions. These cases are probably more common than has been realized.

At least in the patients whose kidneys are pathologic the twitchings or convulsions can be relieved by the administration of a purgative dose of epsom salt by mouth.

SUMMARY AND CONCLUSIONS

1. When normal individuals take epsom salt by mouth they excrete about 40 per cent of the ingested magnesium in the urine in twenty-four hours; but the concentration of magnesium in the blood plasma does not rise appreciably.

2. The concentration of magnesium in the plasma may vary greatly under clinical conditions.

3. There is a clinical syndrome of high plasma magnesium (hypermagnesemia) accompanied by somnolence or coma.

4. This may be induced in patients with renal insufficiency by the oral administration of one or more purgative doses of epsom salt.

5. Many cases of coma in nephritic patients, diagnosed uremic coma, may be simply magnesium coma induced by epsom salt purgation. From such coma patients could probably be awakened by intravenous calcium chloride.

6. Sodium sulphate is preferable to epsom salt for patients with renal insufficiency.

7. There is a clinical syndrome of low plasma magnesium (hypomagnesemia) accompanied by muscular twitching or by convulsions.

8. When this occurs in patients with renal insufficiency, the twitchings or convulsions are relieved by oral administration of epsom salt.

---

13. Denis, W., and Talbot, F. B.: Calcium in the Blood of Children, Am. J. Dis. Child. **21**: 29 (Jan.) 1921.
14. Bulger, H. A., and Gausmann, F.: J. Clin. Investigation **12**: 1135 (Nov.) 1933.
15. Salvesen, H. A., and Linder, G. C.: J. Biol. Chem. **58**: 635 (Dec.) 1924.
16. Lang, K.: Ztschr. f. klin. Med. **122**: 206, 1932.

J. Pharm. Sci., 54(4): 549–554, 1965

Factors Involved in the Browning of Spray-Dried Lactose

By K. T. KOSHY, R. N. DUVALL, A. E. TROUP, and J. W. PYLES

The pH profile of the lactose browning reaction was ascertained with various buffers under accelerated stability conditions and compared to the pH browning profile obtained by similar procedures for both dextrose and galactose. The effect of heat, humidity, and tablet lubricants was studied also. A correlation was found between the ultraviolet absorption spectrum and the 5-hydroxymethylfurfural content of samples of spray-dried lactose. The increase in absorption during the browning process afforded a simple procedure for studying this phenomenon. Also, absorption measurement could serve as a purity test for U.S.P. purposes. A procedure involving the use of anion exchange resins was developed for the purification of spray-dried lactose. This method should be adaptable to large scale commercial processes. Lactose which was spray-dried following this purification treatment was less susceptible to browning than the commercially available material.

FLOW AND COMPRESSIBILITY properties of spray-dried lactose (lactose S.D.) have led to an increased use of this material as an excipient in tablet and capsule formulations. However, the discoloration of such formulations is a problem and has been investigated by various workers. Gunsel and Lachman (1), evaluating the tableting properties of lactose, noted that while lactose S.D. was advantageous in several respects, it darkened more rapidly than conventionally processed lactose. The reason for this has not been clearly established. The significant differences between the two grades are that lactose S.D. contains (*a*) about 8% amorphous lactose, (*b*) slightly higher amounts of other sugars, metals, and ash and (*c*) that it has been exposed to heat during the spray-drying process.

Webb (2) investigated the browning of lactose and other milk products and reported that the hydrogen ion concentration is of major importance and that phosphate buffer has a catalytic effect. However, the reaction was studied over a narrow pH range. Patton and Josephson (3) and Ramsey *et al.* (4) proposed that phosphates may be of secondary importance in the browning of milk products. A synergistic effect of phosphate and protein amino groups has been suggested by Pederson *et al.* (5). Recently, Brownley and Lachman (6) have demonstrated a relationship between the discoloration of lactose S.D. and the presence of free 5-hydroxymethylfurfural (HMF).

The large-scale use of lactose in complex pharmaceutical formulations and the lack of adequate information on the stability of lactose under varying conditions of usage in such systems have prompted this study. This paper deals with some of the factors involved in the browning of lactose in the absence of added amines (referred to hereafter as the normal browning of lactose). Browning which takes place in the presence of added amines will be the subject of a subsequent report. A major portion of this study deals with browning related to changes in the ultraviolet absorption spectrum and HMF content of lactose. The effect of pH on the browning of lactose and on the monosaccharide hydrolysis products, galactose and dextrose, was studied. The effects of heat, humidity, commonly used lubricants, and common buffer species were investigated also. Lactose S.D. was purified by treatment with ion exchange resins and respray-dried in an attempt to improve its resistance to browning.

EXPERIMENTAL

Role of HMF in Lactose Browning.—The ultraviolet absorption spectra of 10% solutions of lactose were determined using a Beckman model DB spectrophotometer with 1-cm. cells and compared to the spectrum of HMF (Aldrich Chemical Co., Inc.). The free HMF and/or related compounds in these samples were determined by adaptation of the reaction with 2-thiobarbituric acid (TBA), described by Keeney and Bassette (7), and applied by Brownley and Lachman for the determination of HMF in lactose (6).

Purification of Spray-Dried Lactose with Ion Exchange Resins.—Aqueous solutions containing 15–18% of lactose S.D. were passed through separate 1 × 22 cm. columns containing the following resins: (*a*) weak anion exchange resin, Amberlite IR-45 (Cl^-), (*b*) Amberlite IR-45 (OH^-), (*c*) strong anion exchange resin, Dowex 1-X4 (Cl^-). The purity of the eluate from each column was monitored by scanning the absorption in the 220–340-mμ region. The eluate was then spray-dried with a Nerco-Niro laboratory model spray-dryer (Nichol-

Received July 28, 1964, from the Corporate Pharmacy Research Laboratory, Miles Laboratories, Inc., Elkhart, Ind.
Accepted for publication December 17, 1964.
Presented to the Scientific Section, A.Ph.A., New York City meeting, August 1964.
The authors thank Mr. L. L. Shankle for technical assistance.
The lactose samples used in this study were supplied by Foremost Dairies, Inc., Appleton, Wis.

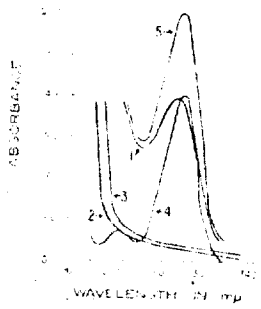


Fig. 1.—Spectra of HMF (4 mcg./ml.) and 10% aqueous solutions of lactose. Key: 1, U.S.P. spray-dried; 2, U.S.P. conventionally processed; 3, analytical reagent grade; 4, HMF; 5, U.S.P. spray-dried with 2 mcg. HMF per milliliter.

Fig. 2.—HMF content by the ultraviolet absorption and TBA methods of a 10% aqueous solution of U.S.P. conventionally processed lactose stored at 80°. Key: 1, HMF by TBA method; 2, HMF by the ultraviolet absorption method.

Engineering and Research Corp.). The respray-dried material was stored under accelerated conditions and its stability compared with that of the raw material by visual observation of resultant browning and by the intensity of the absorption maximum at 275–285 mμ.

pH Profiles of the Dextrose, Galactose, and Lactose Browning Reactions.—Ten per cent solutions of lactose analytical reagent, lactose U.S.P. (conventionally processed), and lactose U.S.P. spray-dried were prepared in buffers of pH 1 through 10. These solutions were stored at 50° and changes in ultraviolet absorption spectra noted at suitable intervals. The relative hydrolysis of lactose in these solutions was determined with reagent strips.[1] The buffer systems were: pH 1, hydrochloric acid–potassium chloride; pH 2–8, citric acid–disodium hydrogen phosphate; pH 8–10, boric acid–sodium hydroxide. The pH profiles of dextrose and galactose also were obtained to ascertain further the significance of hydrolysis as a factor in the browning process.

Catalysis of Buffer Species.—Ten per cent solutions of lactose S.D. were prepared in buffers at pH 4 and 8 with different buffer concentrations at each pH. These solutions were stored at 50° for 1 week, and any change in the absorbance at 276 mμ was noted. The buffer systems were: pH 4, tartaric acid–sodium hydroxide; pH 4, hydrochloric acid–potassium citrate; pH 4, acetic acid–sodium acetate; pH 8, citric acid–disodium hydrogen phosphate.

Effect of Heat and Moisture on Browning.—Samples of lactose S.D. were stored at 50° dry heat, 50° and 70% relative humidity, and room temperature and 70% relative humidity. Ten per cent solutions of each were prepared after 2 weeks of storage and their ultraviolet absorption spectra compared to that of the original.

An 18% aqueous solution of lactose S.D. (containing 5% moisture as loss on drying) was respray-dried with inlet and outlet temperatures of 150–155° and 70–80°, respectively, resulting in a product containing 1.4% moisture. This was stored in sealed containers at elevated temperatures and evaluated visually and by measurement of the absorption at 276 mμ.

Effect of Tablet Lubricants on Browning.—One-gram tablets containing lactose S.D. and 2% of commonly used lubricants were prepared and stored at 50° dry heat in both capped and uncapped bottles and at 50° and 70% relative humidity. At suitable intervals, the tablets were observed visually and the ultraviolet spectra of filtered aqueous solutions determined and compared to the initial spectra of comparable solutions.

DISCUSSION

Role of HMF in Lactose Browning.—Figure 1 shows the typical ultraviolet absorption spectra of aqueous solutions of various grades of lactose, a HMF standard, and the effect of adding HMF to a solution of lactose S.D. All samples of lactose S.D. scanned had an absorption maximum in the region of 275–285 mμ. Solutions of reagent grade and U.S.P. conventionally processed lactose under accelerated conditions developed a peak in this region. For all grades of lactose, the intensity of the peak increased as the solution discolored. This phenomenon was noted recently by Brownley and Lachman (8), who suggested that it might be due to changes in molecular structure. The almost superimposable nature of the absorption curves of lactose S.D. and of HMF and the curve obtained after adding HMF to lactose S.D. solutions, along with other evidence for the presence of this compound in lactose (6), indicated that the absorption peak might be due to HMF and/or closely related compounds. The presence of HMF in concentrates of ethyl ether extracts of solid raw materials was

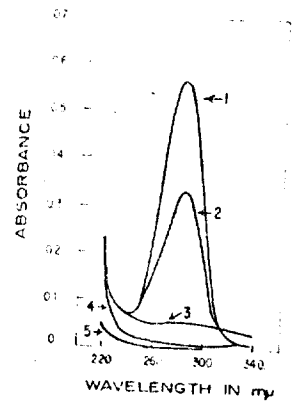


Fig. 3.—Spectra of aqueous solutions of HMF before and after treatment with anion exchange resins; 20 ml. containing 4.4 mcg./ml. passed through 2 Gm. of resin (20–50 mesh) in 1-cm. O.D. column. Key: 1, before treatment; 2, Dowex 1-X4 (Cl^-); 3, Amberlite IR-45 (OH^-); 4, Dowex 1-X4 (OH^-); 5, Amberlite IR-45 (Cl^-).

[1] Clinistix, Ames Co., Inc., Elkhart, Ind.

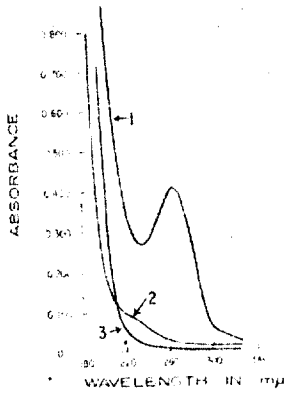


Fig. 4.—Spectra of aqueous solutions of spray-dried lactose before and after treatment with anion exchange resins; 20 ml. of a 10% solution passed through 1 Gm. of resin (20–50 mesh) in 1-cm. O.D. column. Key: 1, before treatment; 2, Dowex 1-X4 (Cl^-) and Amberlite IR-45 (Cl^-); 3, Amberlite IR-45 (OH^-) and Dowex 1-X4 (OH^-).

confirmed by thin-layer chromatography. The concentrates were spotted on silica gel plates, 0.25-mm. thick, and developed with *n*-butyl acetate. HMF was detected by spraying with an aqueous solution made of equal volumes of 0.05 *M* TBA and 40% trichloroacetic acid.

Additional proof for the relationship between the absorbance at 276 mμ and the free HMF content was obtained from the data on a 10% unbuffered aqueous solution of lactose U.S.P. conventionally processed and stored at 80°. The free HMF concentration of this solution was determined daily by the TBA method and from the absorbance at 276 mμ. The data are presented in Fig. 2. Initially, there was no absorbance and no free HMF by the TBA method, but the HMF content increased steadily with time, with a corresponding increase in the absorbance at 276 mμ. The ultraviolet absorption method gave consistently higher values than the TBA method. The reason for this is not known but could be due to the degradation of lactose beyond the HMF stage, resulting in the formation of interfering substances. Since the absorption at 276 mμ increased with browning, it appeared that the intensity of absorption in the region of 275–285 mμ could be used to study this process.

It was felt that if the impurities in lactose S.D. which were strongly suspected to be HMF and/or related compounds could be removed, the resulting material would be less susceptible to browning. Berl and Feazil (9) used a strong anion exchange resin to remove the chromogens from alkaline solutions of a few simple sugars. De Jong and Jansen (10) removed HMF from a starch hydrolyzate with an anion exchange resin partly charged with sulfur dioxide. Kitriene (11) has reported that ion exchange resins could eliminate sulfite treatment in the refining of syrup. Weak and strong anion exchange resins in both their chloride and hydroxyl forms were found to remove HMF from solution, as illustrated in Fig. 3. The strong anion exchange resin in the chloride form was only partially effective, but it was found later that larger quantities of the resin removed HMF completely. Figure 4 shows how effectively the weak and strong anion exchange resins, in both their chloride and hydroxyl forms, removed the chromogens from lactose S.D. Four-hundred milliliters of a 10% solution was passed through a 1 × 22-cm. resin column, and the last portion of eluate still had no absorption in the ultraviolet region.

The free HMF content of 10% solutions of different lots of lactose S.D., lactose U.S.P. conventionally processed, and lactose reagent grade was determined by the TBA method before and after ion exchange treatment. The results presented in Table I confirm the removal of free HMF.

pH Profile of Lactose Browning Reaction.—The effect of pH on the browning of 10% buffered aqueous solutions of lactose is shown in Fig. 5. This pH profile was obtained by measuring the increase in ultraviolet absorbance of solutions stored at 50° for 1 week. In all lactose samples there was a marked increase in absorbance at pH 7 and 8 in phosphate citrate buffer. The curve for lactose S.D. was displaced upward because it had a de-

TABLE I.—FREE HMF CONTENT OF LACTOSE (mcg./Gm.) BY THE TBA METHOD BEFORE AND AFTER ION EXCHANGE TREATMENT[a]

Sample[b]	Before	After[c]
A	5.1	1.0
B	5.5	0.5
C	6.8	1.0
D	5.9	0.5
E	7.8	1.0
F	7.6	1.3
G	4.8	0.2
H	7.8	0.2
I	0.3	0.0
J	1.3	0.0
K	1.2	0.0

[a] Amberlite IR-45 (OH^-). [b] Samples *A* through *H* were spray dried, sample *I* was analytical reagent grade, samples *J* and *K* were U.S.P. conventionally processed. [c] Solutions after ion exchange treatment had negligible ultraviolet absorption in the range of 0–1 mcg./Gm. of HMF.

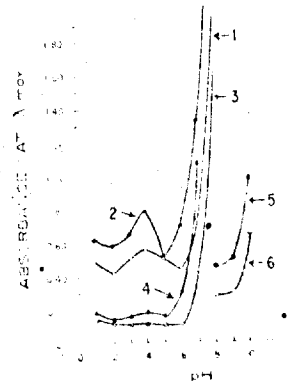


Fig. 5.—pH profile of lactose browning; 10% solutions stored at 50°. Key: 1, U.S.P. spray-dried, 1 week; 2, U.S.P. spray-dried, 2 weeks; 3, analytical reagent grade, 1 week; 4, analytical reagent grade, 2 weeks; 5, U.S.P. spray-dried in borate buffer, 1 week; 6, analytical reagent grade in borate buffer, 1 week. Buffer systems: pH 1, hydrochloric acid–potassium chloride; pH 2–8, citric acid disodium hydrogen phosphate; pH 8–10, boric acid sodium hydroxide.

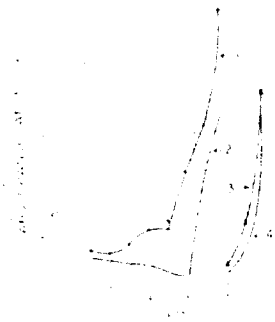


Fig. 6.—pH profile of dextrose and galactose browning: 10% solutions stored at 50° for 1 week. Key: 1, galactose, C.P.; 2, dextrose, analytical reagent; 3, galactose, C.P., in borate buffer; 4, dextrose, analytical reagent, in borate buffer. (Buffer systems the same as in Fig. 5.)

finite peak initially, whereas other grades of lactose did not have such a maximum. The inflection in the curve for lactose S.D. at pH 3–4 was thought at first to be due to experimental error. However, the experiment was repeated, and similar results were obtained. Two-week data were similar, except that the curve was displaced further upward. A slight inflection also was noticeable at this check period at pH 3–4 for both reagent grade and conventionally processed lactose.

Visual observations of the solutions revealed that after 1 week, the solutions at pH 1 were faint yellow, pH 2 through 6 colorless, pH 7 faint yellow, and pH 8 deep yellow. While solutions at pH 2 through 6 were colorless, they exhibited an absorption peak at 275–285 mμ. On prolonged storage, these solutions discolored. It was interesting that, while solutions at pH 8 in phosphate buffer were deep yellow and showed a great increase in absorbance, corresponding solutions in borate buffer were only faintly yellow. Even at pH 9, lactose solutions in borate buffer were discolored only slightly. This protection could be due to complex formation between borate and lactose since many polyhydroxy compounds are known to form complexes with alkali borates or boric acid (12).

One of the reasons for the difference in stability between spray dried and conventionally processed lactose might be the presence of larger quantities of the hydrolytic products, dextrose and galactose, in the former (8). Determination of dextrose in lactose solutions after 4 week at 50° indicated more than 0.5% at pH 1 through 3 and less than 0.25% at pH 4 through 8. Inasmuch as the maximum discoloration and increase in absorbance were observed in solutions of pH 7 and 8 (phosphate–citrate buffer), it is evident that the pH and the buffer species are more significant than the concentration of dextrose and galactose.

The yellow- to brownish-colored material formed in this browning process was extremely water soluble and could not be extracted from aqueous solutions with chloroform, ethyl ether, or *n*-butanol. This general solubility distinguishes the normal browning of lactose from that occurring in the presence of primary amines, evidence of which will be presented in a following communication.

The pH profiles for dextrose and galactose (Fig. 6) were similar to that of lactose, except that galactose discolored over a wider pH range, 5 and above, whereas the other two sugars discolored at pH 7 and above. However, as can be seen from the curves, the relative intensity of the browning of the three sugars was lactose > galactose > dextrose. This also apparently eliminates hydrolysis or the presence of monosaccharides as an important factor in the normal browning of lactose since the extent of browning in a 10% lactose solution is greater than that which would be obtained from the corresponding amounts of monosaccharide hydrolysis products. As in the case of lactose, borate buffer had a protective effect on both monosaccharide browning reactions.

Catalysis of Buffer Species.—Webb (2) demonstrated the catalytic effect of phosphate ions in lactose browning. Figure 7 demonstrates the general base catalytic effect of phosphate, acetate, citrate, and tartrate ions as determined by the increase in absorbance of solutions of lactose S.D. stored at 50° for 1 week. Data in solutions of lower concentration of buffer at pH 8 can be considered only semiquantitative since there was a slight decrease of pH in these solutions. At pH 4, no such change was noticed. It is significant that acetate,

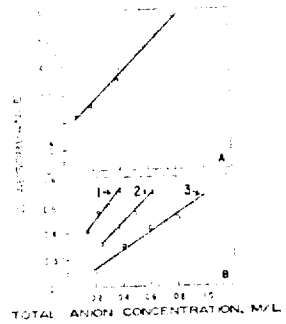


Fig. 7.—Catalytic effect of buffer species on the browning of 10% solutions of spray-dried lactose stored at 50° for 1 week. Key: A, pH 8, citric acid–disodium hydrogen phosphate. B, pH 4; 1, hydrochloric acid–potassium citrate; 2, tartaric acid–sodium hydroxide; 3, acetic acid–sodium acetate.

TABLE II.—EFFECT OF LUBRICANTS ON BROWNING OF SPRAY-DRIED LACTOSE TABLETS[a]

Sample	50° and 70% RH for 2 Mo. A_{278}	50° and 70% RH for 2 Mo. Relative Degree of Browning[c]	50° for 1 Mo. A_{278} Capped Bottles[d]	50° for 1 Mo. A_{278} Uncapped Bottles
Lactose control	1.27	+++	1.04	0.55
Talc A[b]	0.85	++	0.75	0.50
Talc B[b]	0.81	++	0.79	0.49
Mg stearate	0.54	0	0.78	0.51
Ca stearate	0.67	0	0.67	0.56
Starch	0.71	+	0.70	0.63
Na lauryl sulfate	1.25	++++	1.17	0.51

[a] Absorbance measurements were made on filtered 10% aqueous solutions. [b] Aqueous slurries of talcs A and B had a pH of 6.1 and 8.4, respectively. [c] From 0 to ++++ indicates relative increase in browning. [d] All samples had browned noticeably.

Vol. 54, No. 4, April 1965

TABLE III.—COMPARISON OF THE BROWNING OF UNTREATED AND RESIN TREATED U.S.P. SPRAY-DRIED LACTOSE

Sample	Initial Moisture % LOD	Storage Temp., °C.	Relative Degree of Browning[a] Storage Time, Days 0	1	7	14	28
A, raw material	5.0	80	0	++	++	++	+++
B, respray-dried	1.4	80	0	0	0	++	++
C, through Dowex 1-X4 (Cl⁻) and respray-dried	2.4	80	0	0	+	++	++
D, through IR-45(OH⁻) and respray-dried	1.2	80	0	0	0	0	++
A, raw material	5.0	70	0	+	++		
E, sample C with added moisture	5.0	70	0	0	0		
F, sample D with added moisture	5.0	70	0	0	0		
A, raw material	5.0	50	0			0	++
B, respray-dried	1.4	50	0			0	++
C, through Dowex 1-X4 (Cl⁻) and respray-dried	2.4	50	0			0	0
D, through IR-45(OH⁻) and respray-dried	1.2	50	0			0	0

[a] From 0 to +++ indicates relative increases in browning.

citrate, and tartrate ions which are commonly encountered in pharmaceuticals as anions of amine salts, buffer constituents, and as excipients all have a deleterious effect on lactose stability.

Effect of Heat and Humidity on Lactose Browning.—Lactose S.D. powder stored at 50° and 70% relative humidity was discolored markedly, while that at 50° and dry heat was discolored only slightly. The sample at 20° and 70% relative humidity was not discolored. The absorbance values followed the same pattern—namely, highest for the most discolored and no significant change in the sample with no discoloration. These results indicate that a combination of heat and moisture accelerates browning more than either heat or humidity. Thus, if the moisture content of lactose S.D. could be reduced, the tendency for browning could be retarded considerably.

Effect of Tablet Lubricants on Browning.—The effect of lubricants on the browning of lactose tablets is shown in Table II. Results indicate that, except for sodium lauryl sulfate, all other lubricants had some protective effect. One might expect that the alkalinity of the stearate salts would have a deleterious effect since the pH profile showed a general base catalysis. However, the moisture repellent nature of these salts appears to be more important since both magnesium and calcium stearate gave good protection. Sodium lauryl sulfate, which is water soluble, gave no protection against browning, again emphasizing the importance of moisture in this process. This point also was brought out vividly by storing tablets in both capped and uncapped bottles. All tablets in the capped bottles browned noticeably; the corresponding tablets in uncapped bottles, which would not retain the moisture, were similar to controls. The ultraviolet absorption values of filtered aqueous solutions of these tablets agreed with visual observations.

Purification of Spray-Dried Lactose.—Two factors of major significance in the browning process were the presence of HMF and/or related compounds and moisture. It was shown that HMF could be removed effectively by anion exchange resins. Accordingly, 15–20% solutions of lactose S.D. were treated separately with weak and strong anion exchange resins and then respray-dried, as described previously. The stabilities of these samples were compared to those of the raw material (which contained 5% moisture) and raw material respray-dried to a lower moisture level but without ion exchange treatment.

The results presented in Table III clearly demonstrate the effect of moisture and anion exchange resin treatment on the browning of lactose. Lactose S.D. raw material containing 5% moisture discolored after 4 days at 80°. The same material respray-dried to a lower moisture level of 1.4% had not discolored after 7 days. Treatment with Amberlite IR-45 (OH⁻) improved the stability considerably; the material was not discolored even after 14 days. The same effect was noticed at 70°. The three samples at this temperature contained the same amount of moisture. While the untreated raw material discolored after 4 days, there was no change in the resin treated samples after 7 days. Data for the samples stored at 50° demonstrated that, even at lower moisture levels, resin treatment further improved the stability. Samples *B*, *C*, and *D* contained 1.4, 2.4, and 1.2% moisture, respectively. While sample *B* discolored after 28 days, samples *C* and *D*, treated with anion exchange resins, were not discolored.

SUMMARY

1. Some of the factors involved in the browning of lactose in the absence of added amines were studied. Spray-dried lactose was found to have an ultraviolet absorption maximum, whereas U.S.P. conventionally processed and analytical reagent grade lactose did not. A relationship between the intensity of this maximum and the HMF content was shown. This absorbance was related to browning and affords a simple procedure for studying this phenomenon and could serve as a purity test for U.S.P. purposes.

2. The pH profiles of the browning of lactose, dextrose, and galactose were ascertained. Relative order was lactose > galactose > dextrose. The possible presence of monosaccharides does not appear to be a major factor.

3. Browning of lactose was catalyzed by phosphate, tartrate, citrate and acetate ions, whereas borate ions gave remarkable protection.

4. Moisture, in addition to HMF and/or related compounds, was shown to be a major contributory factor.

5. Water repellent lubricants, such as magnesium and calcium stearate, showed good protection against browning of lactose tablets.

6. Treatment with anion exchange resins prior to spray-drying or spray drying to a lower moisture content resulted in a product less susceptible to browning.

REFERENCES

(1) Gunsel, W. C., and Lachman, L., THIS JOURNAL, 52, 178(1963).

(2) Webb, B. H., *J. Dairy Sci.*, 18, 81(1935).

(3) Patton, S., and Josephson, D. V., *ibid.*, 32, 222 (1949); through *Chem. Abstr.*, 45, 276e(1951).

(4) Ramsey, R. J., Tracy, P. H., and Ruehe, H. A., *J. Dairy Sci.*, 16, 17(1933); through *Chem. Abstr.*, 27, 1953 (1933).

(5) Pederson, C. S., Beattie, H. G., and Stotz, E. H., *N. Y. State Agr. Expt. Sta. Bull.*, 728, 3(1947); through *Chem. Abstr.*, 42, 3869c(1948).

(6) Brownley, C. A., Jr., and Lachman, L., THIS JOURNAL, 53, 152(1964).

(7) Keeney, M., and Bassette, R., *J. Dairy Sci.*, 42, 945(1959).

(8) Brownley, C. A., Jr., and Lachman, L., THIS JOURNAL, 52, 86(1963).

(9) Bell, W. G., and Feazel, C. E., *J. Agr. Food Chem.*, 2, 37(1954).

(10) de Jong, G. J., and Jansen, J. C., *Starke*, 9, 147 (1957); through *Chem. Abstr.*, 52, 5864i(1958).

(11) Kitriene, D., *Mokslas ir Tech.*, (No. 3) 33(1960); through *Chem. Abstr.*, 55, 12896i(1961).

(12) Noller, C. R., "Chemistry of Organic Compounds," 2nd ed., W. B. Saunders Co., Philadelphia, Pa., 1957, p.376.

Factors Influencing Percutaneous Absorption II

Absorption of Methyl Ethyl Ketone

By DALE E. WURSTER and ROBERT MUNIES

A method is described for following the percutaneous absorption process with a vapor-phase chromatography technique. Data showing the absorption of methyl ethyl ketone (MEK) from a forearm surface of 91.5 cm.² under both normal and hydrous conditions of the stratum corneum are presented. Hydration of the stratum corneum enhanced the percutaneous absorption rate of methyl ethyl ketone, but this penetrant subsequently partially dehydrated the stratum corneum. The resulting steady-state concentration of MEK in expired air, however, was still higher than that obtained with normal skin.

THE INFLUENCE of hydration of the stratum corneum on the percutaneous absorption of various salicylate esters in humans was studied previously (1). This study showed that the actual absorption rate expressed in terms of moles cm.$^{-2}$ hr.$^{-1}$ could be increased several fold by the hydration of this tissue. The magnitude of this increase was shown to be related to the oil/water distribution coefficient and the water solubility of these chemically similar salicylate esters. In general, the absorption of the more water-soluble compounds was enhanced more greatly by the hydration effect, but the precise relationship between the physical constants of the chemical and percutaneous absorption remains obscure. However, the single but strong influence of skin hydration on percutaneous absorption now has been shown to occur with several chemical agents including ethyl nicotinate (2), naphazoline (3, 5), steroids (4), aniline (6, 7), benzidine, dichlorobenzidine, dianisidine, and *o*-toluidine (8).

This paper is thus a part of a larger study in which an attempt will be made to elucidate relationships between the percutaneous absorption rate, the functional groups, and physical constants of the absorbed chemical.

Received August 26, 1964, from the School of Pharmacy, University of Wisconsin, Madison.
Accepted for publication December 3, 1964.
Presented to the Scientific Section, A.PH.A., New York City meeting, August 1964.
Abstracted in part from a dissertation submitted by Robert Munies to the Graduate School, University of Wisconsin, Madison, in partial fulfillment of Doctor of Philosophy degree requirements.
This investigation was supported in part by the University of Wisconsin Research Committee, Vick Division, Research, Mount Vernon, N. Y., and the Malstrom Chemical Corp., Newark, N. J.

METHOD

A liquid chemical compound of simple structure was desired to eliminate the complicating factors involved in the release of the drug from the physical system. Additional requirements for the penetrant indicated that it should be nonirritating, have low systemic toxicity, and be excreted unchanged mainly *via* one pathway. Because of the difficulty in analyzing excretions and body fluids for the absorbed compound, a substance which possessed a high vapor pressure and which could be detected easily in the expired air by vapor phase chromatography was desired also. Methyl ethyl ketone (MEK) appeared to meet most of the above requirements.

To simplify working with the complex biological system further, the study was designed so that

A Contribution to the Study of Hypnotics with Paralyzing Action on the Thalamic Centers. IV. With regard to the effect of Magnesium Sulfate on the External Secretion of the Pancreas

Jean La Barre and Olga Vesselovsky

Arch. Internat. de Pharmacodyn. et de Therap. 67: 267-273, 1942

In a preceding study[1] we reported that the barbiturate derivatives such as veronal, dial and nembutal besides their stimulant effect can induce a marked inhibition of the external secretion of the pancreas even though their action is localized in the isolated central nervous system. On the other hand, magnesium sulfate whose hypnotic effects, all similar to those of barbiturates, are attributed to a paralysis of the thalamic zone, induces a reduction and sometimes a complete suppression of postinsulin hypercontractility and gastric hypersecretion[2]. These phenomenon appear to be due to an inhibition of gastric secretion by the central nervous system rather than cellular in origin.

Continuing our research on the action of hypnotics on the digestive glands, in this study we have tried to determine the influence of magnesium sulfate on the functioning of the exocrine portion of the pancreas.

H. W. Gantt and G. V. Volborth[3] have previously reported that at the time of the intraduodenal administration of this material a slight increase of pancreatic secretion is produced. Magnago[4] observed with man under these conditions, that there was a marked secretory stimulation accompanied by an increase in the trypsin content of the duodenal juice. According to H. Grunbers[5], intravenous injection of magnesium salts in doses varying between 100-200 mg/kg brings about a marked glandular

hyperfunction attributable to the direct effect of this material on the pancreatic cells.

In the present research we have been interested in studying the effect of magnesium sulfate either on dogs kept constantly secreting by the continuous intrasaphenous injection of a diluted secretin solution, or by means of two repeated stimulations by administering a test dose of secretin both before and after injecting the hypnotic material.

I. The Effect of Magnesium Sulfate on Pancreatic Secretion Induced by the Continuous Administration of Secretin.

In a first series of experiments, utilising the procedure previously described for the injection of secretin[1], we used chloralosed dogs which had undergone before the fistulization of Wirsung's canal, ligature of the pyloric mucosa in order to prevent the passage of chyme acid into the duodenal region, in the course of the experiment.

When the secretion becomes stable the animal is placed under artificial respiration and we proceed with the intrasaphenous injection of 100-150 mg of magnesium sulfate dissolved in 20 cc of distilled water.

The results of 4 experiments carried out in this way can be found in Table 1.

The variation in pancreatic excretion under the influence of a 150 mg/kg dose of magnesium sulfate can be followed in Figure 1. In I, we show the curve of a control study.

Thus by examining the values in Table 1, one sees that in most instances, under the influence of weak doses of magnesium sulfate (100 mg/kg) there is a slight stimulation of brief duration in pancreatic excretion. At more elevated concentrations, there is a marked reduction in the amount of secreted pancreatic juice. (Curve II, Figure 1.)

The lipase content of the juice varies little when compared to the amount secreted per cc. If the amount of enzymes emitted during a quarter of an hour of observation is totaled up, in most instances, the enzyme content varies in proportion to the quantity of juice secreted during the same time period.

In animals in which the sensitivity of the pancreatic gland is increased by the continuous administration of secretin, these studies show that average doses (150 mg) of magnesium sulfate result in a moderation of the external secretion of the pancreas. This secretory diminution is given some perspective by the fact that in experimental doses, magnesium sulfate decreases the excitability of the central nervous system.

II. The Effect of Magnesium Sulfate on the Pancreatic Secretion Induced by Administration of two Successive Doses of Secretin.

In a second series of studies, our first finding was that when two injections of 10 mg/kg secretin are given at 60 minute intervals to chloralosed dogs with a pancreatic fistula, it is observed that the secretory responses in the same animal appear to be quite constant.

Consequently, it seemed of interest to investigate if after administering 100-150 mg of magnesium sulfate one would find appreciable modifications of the pancreatic excretion induced by an intrasaphenous injection of a test dose of secretin.

Under these conditions, it is found (Table 2 and Figure 2) that for weak doses of magnesium sulfate (100 mg) the pancreatic secretory response is not particularly modified under the influence of this substance (Table 2, exp. A). For concentrations reaching 150 mg one sees during

the second experiment, either no modification (Table 2, exp. B) or a definite diminution of the secretory faculty of the gland (Table 2, exp. C and D).

With regard to the enzyme strength of the collected juice, in most cases a reduction in lipase content is found, calculated from the total amount of secretion emitted in the course of the experiment (Table 2).

III. Causes of the Inhibition of Pancreatic Secretion Following the Administration of Magnesium Sulfate.

In order to better understand the origin of the decrease in pancreatic secretory ability after administration of magnesium sulfate, we carried out a third series of experiments on vagotomised dogs with a sectioned medulla at the level of the third cervical vertebra. The animal was kept alive by artifical respiration and we did not begin the experiment until about 1 hour after the section of the medulla. After we affirmed that it was possible to obtain a constant pancreatic secretion with decapitated dogs, we studied the influence of magnesium sulfate at a dose of 100-150 mg/kg on an animal in which the pancreas was kept continually secreting by intravenous administration of secretin.

In the absence of the superior central nervous system (encephalic and bulbus) either a postmagnesium stimulation is produced or the pancreatic secretion is maintained at its initial value.

Carrying out these experiments by studying dogs with sectioned medullas shows that under the influence of magnesium sulfate, the secretory reactions produced by two successive injections of duodenal hormone no longer occur by blocking the postsecretin pancreatic hypersecretion. Of the

four experiments carried out in this way, in two of the cases the secretory response from the administration of duodenal hormone was not modified (Figure 3, curve IA, control experiment and curve IB, after magnesium sulfate administration). In the two other examples we observed a slight increase in the pancreatic secretion (Figure 3, curves IIA and IIB).

Finally, the results of these experiments appear to be that if the pancreas, is removed from the influence of the central nervous system by a section of the upper medulla, the depressive action of magnesium sulfate on the acinarious function is not seen.

General considerations

It follows from the research that we have just cited that magnesium sulfate has a depressive effect on gastric and pancreatic secretions. We have in fact established that the inhibition of postinsulin gastric hypersecretion can be attributed to a postmagnesium paralysis of the thalamic centers. With regard to the exocrine function of the pancreas, the observed blocking phenomenon appears to also depend on the integrity of the encephalic centers. In fact, in the absence of the control of the central nervous system the postmagnesium reduction of pancreatic excretion is no longer produced.

Just like the opiate hypnotics, strong doses of magnesium sulfate (100-150 mg/kg) have the disadvantage of reducing the production of pancreatic juice and the formation of enzymes in persons in which this important function is cut-off by administering secretin or by the presence of food in the digestive tract.

Summary

1. Magnesium sulfate in 150-200 mg doses causes a marked moderation in pancreatic excretion which has been actively maintained by continuous intravenous administration of a dilute secretin solution.

2. The same secretory reduction is observed when the procedure followed is two repeated excitations by injecting the same dose used of excretin both before and 10 minutes after the administration of this hypnotic material.

3. The enzyme strength of the collected juice shows a reduction in lipase content evaluated by summing the amount of enzymes emitted in the course of the experiment.

4. The postmagnesium inhibition of pancreatic secretory ability no longer occurs after an upper medullary section in vagotomised dogs. This phenomenon therefore appears to have its origin in the central nervous system.

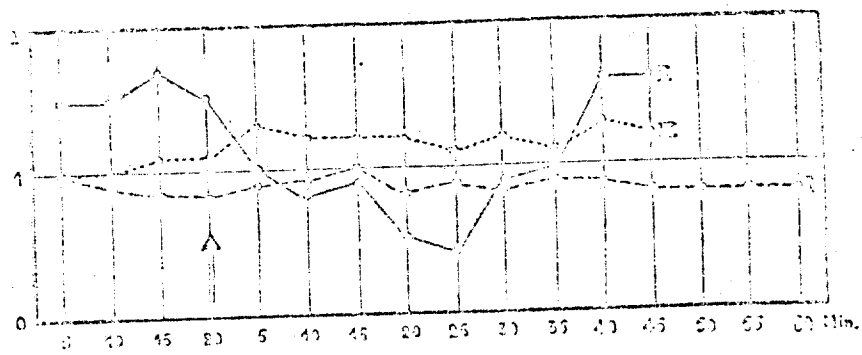


Fig. 1. Changes in the pancreatic secretion under the influence of an injection of magnesium sulfate (150 mg).

Line I: The curve representing a control study.

Line II: The curve respresenting secretory changes after injection of 150 mg/kg of magnesium sulfate.

Line III: The curve representing the secretory variations of a dog with a medullary section which received 150 mg/kg magnesium sulfate.

Ordinate: cc of secreted juice

Abscissa: time in minutes

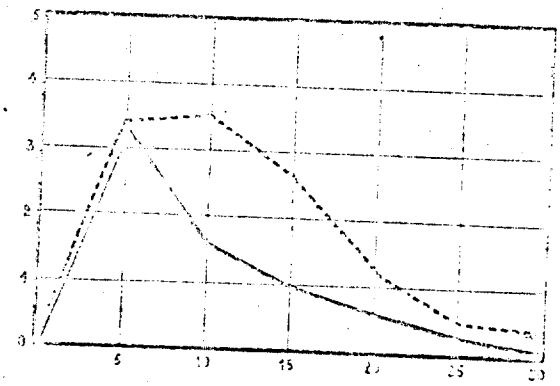


Fig. 2. Secretin experiment in a dog which has received 150 mg of magnesium sulfate.

Broken line: Curve representing the pancreatic secretion in the course of the control experiment.

Smooth line: Curve representing the pancreatic response under the influence of magnesium sulfate.

Ordinate: cc of secreted juice

Abscissa: time in minutes

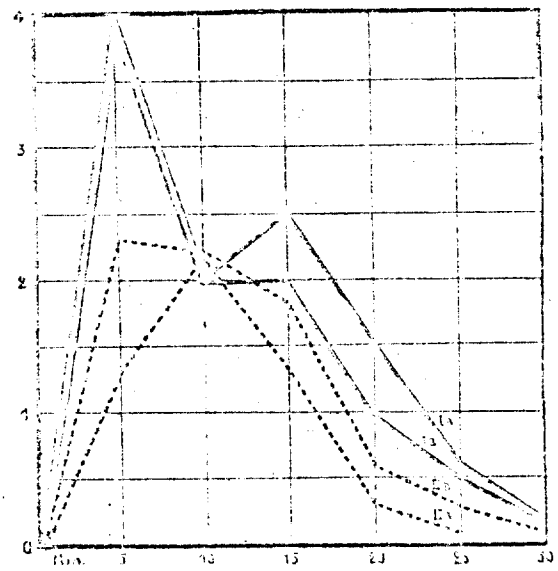


Fig. 3. Changes in the pancreatic secretion in vagotomised dogs with sectioned medullas receiving two successive injections of 10 mg of secretin.

Curve IA: Control experiment.

Curve IB: After administration of 150 mg of $MgSO_4$.

Curve IIA: Control experiment.

Curve IIB: After administration of 150 mg of $MgSO_4$.

Ordinate: Amount of secreted juice in cc.

Abscissa: Time in minutes.

Table 1

Continuous Secretin Injection

Time of sampling	cc of Pancreatic juice	Lipase at time of discoloration	Lipase in units of cc	Total lipase in units of about 5 min.
		Experiment A		
5	1.5			
10	1.5			
15	1.5	45	315	4.725
20	1.3	42	335	4.355
25	1.2	45	315	3.780
		Injection of 100 mg of $MgSO_4$		
5	1.1	45	315	3.465
10	1.8	45	315	5.670
15	0.8	45	315	2.520
20	0.9	45	315	2.835
25	1.0	--	---	--
30	0.8	40	350	2.800
35	0.8	--	---	--
40	1.0	40	350	3.500
45	---	--	---	--
50	---	--	---	--

Table 1 (continued)

Continuous Secretin Injection

Time of sampling	cc of Pancreatic juice	Lipase at time of discoloration	Lipase in units of cc	Total lipase in units of about 5 min.
		Experiment B		
5	---	--	---	--
10	0.6	--	---	--
15	0.5	25	675	3.375
20	0.5	28	575	2.875
25	0.4	29	550	2.200
		Injection of 100 mg of $MgSO_4$		
5	0.8	22.5	750	6.000
10	0.4	--	---	--
15	0.3	31	510	1.530
20	0.4	--	---	--
25	0.4	31	510	2.040
30	0.3	--	---	--
35	0.3	30	525	1.575
40	0.3	--	---	--
45	0.3	28	575	1.725
50	0.3	--	---	--

Table 1 (continued)

Continuous Secretin Injection

Time of sampling	cc of Pancreatic juice	Lipase at time of discoloration	Lipase in units of cc	Total lipase in units of about 5 min.
		Experiment C		
5	---	--	---	--
10	1.5	--	---	--
15	1.5	28	575	8.625
20	1.7	--	---	--
25	1.5	25	675	10.125
		Injection of 100 mg of $MgSO_4$		
5	1.0	18	970	9.700
10	0.8	--	---	--
15	0.9	17	1000	9.000
20	0.5	--	---	--
25	0.4	17	1000	4.000
30	0.9	--	---	--
35	1.0	15	1050	10.500
40	1.6	--	---	--
45	---	--	---	--
50	---	--	---	--

Table 1 (continued)

Continuous Secretin Injection

Time of sampling	cc of Pancreatic juice	Lipase at time of discoloration	Lipase in units of cc	Total lipase in units of about 5 min.
		Experiment D		
5	---	--	---	--
10	1.1	--	---	--
15	1.8	--	---	--
20	1.4	55	230	3.220
25	1.8	55	230	4.140
		Injection of 100 mg of $MgSO_4$		
5	1.7	55	230	3.910
10	1.7	--	---	--
15	1.5	61	190	2.850
20	1.2	--	---	--
25	1.1	55	230	2.530
30	1.1	--	---	--
35	1.0	41	340	3.400
40	0.9	--	---	--
45	---	--	---	--
50	---	--	---	--

Table 2

Time of sampling	cc of Pancreatic juice	Lipase at time of discoloration	Lipase in units of cc	Total lipase in units of about 5 min.
		Experiment A		
		Injection of 10 mg of secretin (per kg)		
5	1.1	18.5	950	10.450
10	2.2	35.5	450	9.900
15	1.7	50	265	4.500
20	0.7	40	350	2.450
25	0.2	32	500	1.000
30	0.1	24	700	.700
	6.0			29.000
		Injection of 100 mg of $MgSO_4$ then, 10 minutes after injection of 10 mg of secretin (per kg)		
5	2.2	18.5	950	10.900
10	1.8	38.5	375	6.750
15	1.4	65	175	2.450
20	0.5	60	200	1.000
25	0.2	50	265	.530
30	0.1	37	400	.400
	6.2			22.000

Table 2 (continued)

Time of sampling	cc of Pancreatic juice	Lipase at time of discoloration	Lipase in units of cc	Total lipase in units of about 5 min.
		Experiment B		
		Injection of 10 mg of secretin (per kg)		
5	4.7	14	1100	51.700
10	4.3	37.5	390	16.770
15	2.5	1 hr. 11	145	3.625
20	2.2	1 hr. 12	140	3.080
25	2.2	1 hr. 8	160	3.520
30	0.7	56	220	1.540
	16.6			80.200
		Injection of 100 mg of $MgSO_4$ then, 10 minutes after injection of 10 mg of secretin (per kg)		
5	2.2	18.5	950	10.900
10	1.8	38.5	375	6.750
15	1.4	65	175	2.450
20	0.5	60	200	1.000
25	0.2	50	265	.530
30	0.1	37	400	.400
	6.2			22.000

Table 2 (continued)

Time of sampling	cc of Pancreatic juice	Lipase at time of discoloration	Lipase in units of cc	Total lipase in units of about 5 min.
		Experiment C		
		Injection of 10 mg of secretin (per kg)		
5	3.4	18.5	950	32.300
10	3.5	39.5	360	12.600
15	2.6	41	340	8.840
20	1.2	35	440	5.280
25	0.5	32	500	2.500
30	0.4	32	500	2.000
	11.6			63.500
		Injection of 100 mg of $MgSO_4$ then, 10 minutes after injection of 10 mg of secretin (per kg)		
5	3.3	18.5	950	31.350
10	1.6	31'40"	510	8.160
15	1.0	33	480	4.800
20	0.6	29	550	3.300
25	0.3	28	575	1.725
30	0.1	27	---	1.200
	6.9			50.550

Table 2 (continued)

Time of sampling	cc of Pancreatic juice	Lipase at time of discoloration	Lipase in units of cc	Total lipase in units of about 5 min.
		Experiment D		
		Injection of 10 mg of secretin (per kg)		
5	3.5	18.5	950	33.250
10	2.0	29	550	11.000
15	1.9	41	340	6.460
20	2.1	38.5	375	7.875
25	0.8	34	460	3.680
30	0.6	29	550	3.300
	10.9			65.400
		Injection of 100 mg of $MgSO_4$ then, 10 minutes after injection of 10 mg of secretin (per kg)		
5	3.1	17	1000	31.000
10	1.2	29	550	6.600
15	1.3	36.5	410	3.330
20	0.9	34.5	450	4.050
25	0.4	30	525	2.100
30	0.3	25.5	650	1.950
	7.2			51.000

Arch. Int. Pharmacodyn., 1942, LXVII, Fs 3. 267

TRAVAUX DU LABORATOIRE DE PHARMACODYNAMIE DE L'UNIVERSITÉ DE BRUXELLES. DIRECTEUR : PROF. DR. J. LA BARRE

CONTRIBUTION A L'ÉTUDE DES SELS NOTIQUES À ACTION PARTICULIÈRE SUR LES CENTRES THALAMIQUES

IV. A propos de l'action du sulfate magnésique sur la sécrétion externe du pancréas.

PAR

JEAN LA BARRE ET OLGA VESSELOVSKY

(Travail reçu le 9-1-1942)

Dans une précédente étude (1), nous avons signalé que les dérivés barbituriques tels que le veronal, le dial et le nembutal peuvent provoquer, à côté de leur effet stimulant sur la sécrétion externe du pancréas, une inhibition marquée lorsqu'on localise leur action sur certains noyaux nerveux isolés. D'autre part, le sulfate magnésique dont les effets hypnotiques sont, tout comme pour les dérivés barbituriques, attribués à une paralysie des zones thalamiques, provoque une atténuation et même parfois une suppression complète de l'hypercontractilité et de l'hypersécrétion gastrique postinsulinique (2). Ces phénomènes semblent devoir être attribués à une inhibition nerveuse centrale et non cellulaire de l'excrétion gastrique.

Poursuivant nos recherches à propos de l'action des hypnotiques sur les glandes digestives, nous avons cherché dans ce travail à déterminer l'influence du sulfate magnésique sur le fonctionnement de la portion exocrine du pancréas.

H. W. Cavett et C. V. Volborth (3) ont précédemment signalé que lors de l'administration intraduodénale de cette substance il se produisait une faible augmentation de la sécrétion pancréatique. Chez l'Homme, Magnago (4) observait dans ces conditions une stimulation sécrétoire très marquée accompagnée d'un accroissement de la teneur du suc duodénal en trypsine. D'après H. Grünberg (5), l'injection

intraveineuse de sels magnésiques, aux doses variant entre 100 et 200 mg. par kg. entraînerait un hyperfonctionnement glandulaire marqué attribuable à une action directe de cette substance sur les cellules pancréatiques.

Dans les présentes recherches, nous nous sommes attachés à étudier l'action du sulfate magnésique, soit chez des chiens maintenus en sécrétion constante par l'injection intracaphène continue d'une solution de sécrétine diluée, soit en procédant à deux stimulations répétées par administration d'une dose test de sécrétine pratiquée avant et après l'injection de cette substance hypnotique.

I. — *Effets du sulfate magnésique sur la sécrétion pancréatique provoquée par administration continue de sécrétine*

En utilisant pour l'injection de sécrétine le dispositif précédemment décrit (1), dans une première série d'expériences, nous avons employé des chiens chloralosés ayant subi avant la fistulisation du canal de Wirsung une ligature de la muqueuse pylorique afin d'empêcher le passage du chyme acide dans la région duodénale, au cours de l'essai.

Lorsque la sécrétion devient stable nous plaçons l'animal sous respiration artificielle et nous procédons à l'injection intraveineuse de 100 à 150 mg. de sulfate magnésique dissous dans 20 cc. d'eau distillée.

On trouvera dans le tableau I les résultats de 4 expériences ainsi conduites.

Dans la figure 1, on peut suivre les variations de l'excrétion pancréatique sous l'influence de l'administration d'une dose de 150 mg. par kg. de sulfate magnésique. En I, nous représentons la courbe d'un essai témoin.

Ainsi qu'on peut s'en rendre compte à l'examen de ces valeurs (TABLEAU I), sous l'effet de faibles doses de sulfate magnésique (100 mg. par kg.) on assiste dans la plupart des cas à une stimulation légère et de courte durée de l'excrétion pancréatique. Pour des concentrations plus élevées (150 mg.) il existe une modération marquée de la quantité de suc sécrété. (Courbe II, Fig. I).

La teneur en lipase du suc varie peu si on la rapporte à la quantité sécrétée par cc. Si l'on totalise la quantité de ferments émis pendant un quart d'heure d'observation, dans la majorité des cas, la teneur en enzymes varie proportionnellement à la quantité de suc sécrété pendant un même laps de temps.

Il résulte de ces essais que le sulfate magnésique [illegible] à doses moyennes (150 mg.) une modération de la sécrétion pancréatique

TABLEAU I

Injection continue de sécrétine

Moment du prélèvement	Lipase cc. du suc pancréatique brut	Lipase en minutes de décoloration	Lipase en unités par cc.	Lipase total en unités par 5 minutes	Lipase cc. du suc pancréatique brut	Lipase en minutes de décoloration	Lipase en unités par cc.	Lipase total en unités par 5 minutes	Lipase cc. du suc pancréatique brut	Lipase en minutes de décoloration	Lipase en unités par cc.	Lipase total en unités par 5 minutes	Lipase cc. du suc pancréatique brut	Lipase en minutes de décoloration	Lipase en unités par cc.	Lipase total en unités par 5 minutes
	Expérience A				*Expérience B*				*Expérience C*				*Expérience D*			
													Injection de 100 mg. de SO_4Mg			
5'	1,5	45'	315	4.725	0,6	25	675	3.375	1,5	25'	575	8.625	1,1	55	500	5.500
10'	1,5	45'	315	4.725	0,5	28'	575	2.875	1,5	—	—	—	1,2	—	—	—
15'	1,6	42'	335	4.355	0,5	29'	550	2.750	1,7	—	—	—	1,2	61'	500	2.800
20'	1,3	48'	315	3.780	0,4	—	—	—	1,5	25'	675	10.125	1,1	55	500	2.500
25'	1,2												1,1	—	—	—
													1,0	47'	300	3.100
													0,9	—	—	—
	Injection de 100 mg. de SO_4Mg								*Injection de 150 mg. de SO_4Mg*				*Injection de 150 mg. de SO_4Mg*			
5'	1,1	45'	315	3.495	0,8	22'30"	750	6.000	1,0	15'	675	9.000	1,1	55	500	5.500
10'	1,8	45'	315	5.670	0,4	—	—	—	0,8	—	—	—	1,2	—	—	—
15'	0,8	42'	315	2.520	0,3	31'	510	1.530	0,9	17'	1000	9.000	1,5	61'	500	2.800
20'	0,9	45'	315	2.835	0,4	—	—	—	0,5	—	—	—	1,3	—	—	—
25'	1,0	—	—	—	0,4	31'	510	2.040	0,4	17'	1000	4.000	1,1	55	500	2.500
30'	0,8	49	330	2.800	0,3	—	—	—	0,6	—	—	—	1,1	—	—	—
35'	0,8	—	—	—	0,3	30'	525	1.575	1,0	15'	1000	10.000	1,0	47'	300	3.100
40'	1,0	49'	330	3.500	0,3	—	—	—	1,0	—	—	—	0,9	—	—	—
45'					0,3	28'	575	1.725								
50'					0,3	—	—	—								

…z les animaux dont la sensibilité de la glande pancréatique est exagérée …e l'administration continue de sécrétine. Cette diminution sécrétoire

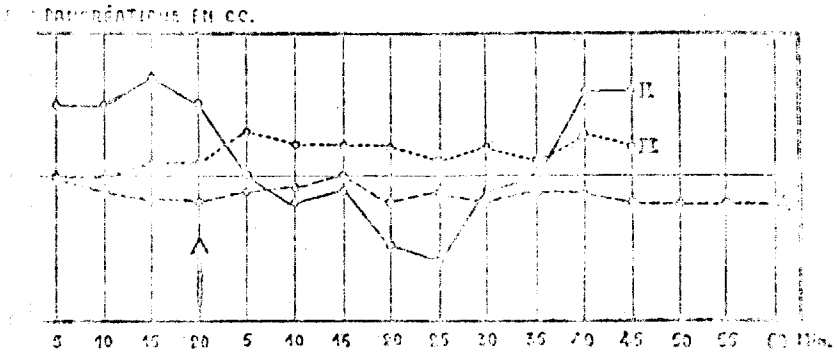


FIG. 1

Variations de la sécrétion pancréatique sous l'influence de l'injection de sulfate …gnésique (150 mg.).
En I : courbe représentant un essai témoin.
En II : courbe représentant les variations sécrétoires après injection de 150 mg. de sulfate magnésique par kg.
En III : courbe représentant les variations sécrétoires d'un chien à moelle sectionnée ayant reçu 150 mg. de sulfate magnésique par kg.
En ordonnées : cc. de suc sécrété.
En abscisses : temps en minutes.

…pourrait être mise en relation avec le fait que le sulfate magnésique …diminue l'excitabilité du système nerveux central aux doses expéri…mentées.

2. — *Influence du sulfate magnésique sur la sécrétion pancréatique provoquée par l'administration de deux doses successives de sécrétine*

Dans une deuxième série d'essais, nous avons constaté tout d'abord …que lorsqu'on pratique à 60 minutes d'intervalle deux injections de …0 mg. de sécrétine par kg. à des chiens chloralosés porteurs d'une …tule pancréatique, on note des réponses sécrétoires présentant une …onstance suffisante, chez un même animal.

Dès lors, il convenait d'examiner si après l'administration de 100 …150 mg. de sulfate magnésique on observerait des modifications …préciables de l'excrétion pancréatique provoquée par l'injection …tra-veineuse d'une dose test de sécrétine.

Dans ces conditions, on note (TABLEAU II et FIGURE 2) que pour …s faibles doses de sulfate magnésique (100 mg.) la réponse sécrétoire

TABLEAU II

Minutes après l'injection de sécrétine	Lipase en cc. de suc pancréatique	Lipase en mil. mites de dédoublement	Lipase en unités par cc.	Lipase totale en unités par 5 minutes	Lipase en cc. de suc pancréatique	Lipase en mil. mites de dédoublement	Lipase en unités par cc.	Lipase totale en unités par 5 minutes	Lipase en cc. de suc pancréatique	Lipase en mil. mites de dédoublement	Lipase en unités par cc.	Lipase totale en unités par 5 minutes	Lipase en cc. de suc pancréatique	Lipase en mil. mites de dédoublement	Lipase en unités par cc.	Lipase totale en unités par 5 minutes
	Expérience A				*Expérience B*				*Expérience C*				*Expérience D*			
	Injection de 100 mg. de SO_4Mg				Injection de 10 mg. de sécrétine par kg.											
5'	1.1	18'30''	950	10.450	4.7	14'	1100	51.700	3.4	16'30''	956	32.500	3.5	18'30''	950	33.250
10	2.8	35'30''	450	9.000	4.3	37'30''	390	16.770	3.5	39'30''	360	12.600	2.6	2'	500	11.000
15	1.7	50'	265	4.500	2.5	1h. 1'	145	3.625	2.6	41'	340	8.840	1.6	41'	340	6.800
20	0.7	40	350	2.450	2.2	1h. 12'	140	3.080	1.2	35'	440	5.280	0.7	1h.30'	275	2.475
25	0.2	35'	500	1.000	2.2	1h. 8'	160	3.520	0.3	32'	500	2.500	0.3	1h.	400	1.200
30	0.1	21'	700	700	0.7	26'	220	1.540	0.4	32'	500	2.000	0.4	29'	550	3.300
	6.6			23.600	16.6			80.000	11.6			63.500	7.0			65.400
	Injection de 150 mg. de SO_4Mg				*Injection de 150 mg. de SO_4Mg*				*Injection de 150 mg. de SO_4Mg*				*Injection de 150 mg. de SO_4Mg*			
	puis 10 minutes après injection de 10 mg. de sécrétine (par kg.)															
5'	2.2	18'30''	950	10.000	4.0	13'	1150	46.000	3.3	18'30''	950	31.350	2.1	15	1000	21.000
10	1.8	38'30''	375	6.750	3.2	31'40''	510	16.320	1.6	31'40''	510	8.160	1.2	20'	500	6.000
15	1.4	65'	175	2.450	2.6	1h. 6'	170	4.420	1.0	33	470	4.800	1.1	30'30	410	4.400
20	0.5	60'	200	1.000	2.4	1h. 17'	115	2.760	0.6	29'	550	3.300	0.9	38'30	510	4.600
25	0.1	50	265	500	2.1	1h. 12'	130	2.730	0.3	25'	575	1.725	0.5	35'	575	2.300
30	0.1	35	400	400	1.5	1h. 12'	135	2.025	0.1	27'		1.200	0.3	33'30	600	1.800
	4.1			22.000	16.0			74.000	6.0			50.500	7.0			51.000

pancréatique ne se modifie pas notablement sous l'influence de cette substance (TABLEAU II, exp. A). Pour des concentrations atteignant 150 mg., on observe lors de la seconde épreuve, soit aucune modification (TABLEAU II, exp. B), soit une diminution nette de la faculté sécrétoire de la glande (TABLEAU II, exp. C et D).

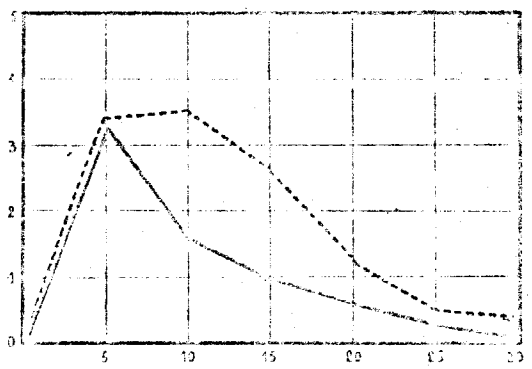


FIG. 2

Épreuve sécrétinique chez un chien ayant reçu 150 mg. de sulfate magnésique.

En trait interrompu : courbe représentative de la sécrétion pancréatique au cours de l'épreuve témoin.

En trait plein : courbe indiquant la réponse pancréatique sous l'influence du sulfate magnésique.

En ordonnée : cc. de suc sécrété.

En abscisses : temps en minutes.

Pour ce qui concerne le pouvoir fermentaire du suc recueilli, on constate dans la majorité des cas une diminution de la teneur en lipase évaluée d'après la quantité totale de sécrétion émise au cours de l'épreuve (TABLEAU II).

3. — *Causes de l'inhibition sécrétoire pancréatique consécutive à l'administration de sulfate magnésique*

Afin de mieux comprendre l'origine de la diminution des facultés sécrétoires du pancréas après administration de sulfate magnésique, nous avons réalisé une troisième série d'expériences sur des chiens vasomotisés à moelle sectionnée au niveau de la troisième vertèbre cervicale. L'animal est entretenu en vie par la respiration artificielle et nous ne commençons l'expérience qu'environ 1 heure après la section de la moelle. Après nous être assuré qu'il était possible d'obtenir une sécrétion pancréatique constante chez ces chiens [illegible]

[illegible] influence du sulfate magnésique à la dose de 100 à 150 mg. (par kg.) sur un animal dont les sécrétions variaient en sécrétion continue par administration intraveineuse de sécrétine.

En Fig. 3, nous avons tracé les courbes [illegible] (encéphale et bulbe). Il [illegible] une sécrétion pancréatique [illegible], tout le [illegible] de la sécrétion pancréatique [illegible] (courbe III de la Fig. 3).

[illegible] chez les chiens à moelle sectionnée, [illegible] d'hormones [illegible] montrent que sous l'influence du sulfate magnésique il ne s'en plus produit de [illegible] de l'hyposécrétion pancréatique post-cervicale. Dans des quatre expériences ainsi [illegible], dans deux cas la réponse sécrétoire à l'administration de l'hormone [illegible] ne s'est pas trouvée modifiée (courbes I A (essai témoin) et I B (essai après administration de sulfate magnésique), FIG. 3). Dans deux autres exemples, nous avons observé une exagération légère de la sécrétion pancréatique (courbes II A et II B, FIG. 3).

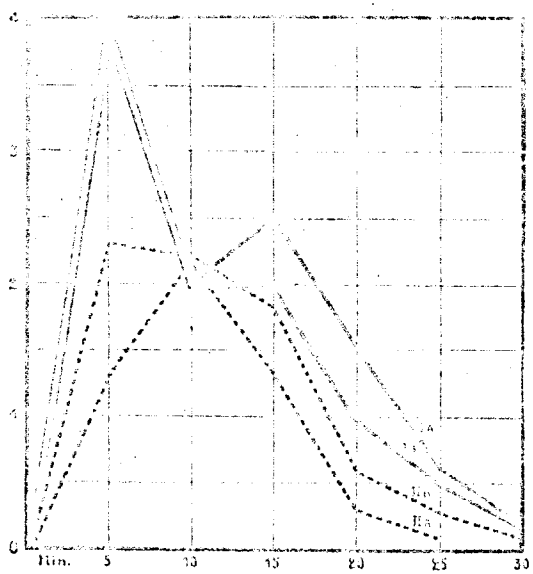


FIG. 3

Variations de la sécrétion pancréatique chez des chiens vagotomisés à moelle sectionnée, recevant deux injections successives de 10 mg. de sécrétine.

Courbe IA : essai témoin.

Courbe IB : après administration de 150 mg. de SO_4Mg.

Courbe IIA : essai témoin.

Courbe IIB : après administration de 150 mg. de SO_4Mg.

En ordonnée : quantité de suc sécrété en cc.

En abscisse : temps en minutes.

En définitive, il semble résulter de ces expériences que si l'on soustrait le pancréas à l'influence du système nerveux central par une section médullaire haute, on n'observe plus l'action dépressive du sulfate magnésique sur la fonction acinaire.

Considérations générales

Il résulte des recherches que nous avons précédemment relatées que le sulfate magnésique possède une action dépressive sur les sécrétions gastrique et pancréatique. Nous avions en effet constaté que l'inhibition de l'hypersécrétion gastrique particulière devait être attribuée à une paralysie postmagnésienne des centres thalamiques. Pour ce qui concerne la fonction exocrine du pancréas, les phénomènes inhibiteurs observés semblent également dépendre de l'intégrité des centres encéphaliques. En effet, en l'absence du contrôle nerveux central il ne se produit plus de diminution postmagnésienne de l'excrétion pancréatique.

Tout comme les hypnotiques opiacés, le sulfate magnésique, à fortes doses (100 à 150 mg. par kg.), présente l'inconvénient d'atténuer la production du suc pancréatique et la formation des enzymes chez les sujets dont cette importante fonction est déclenchée par l'administration de sécrétine ou par la présence d'aliments dans le tube digestif.

Résumé

1° Le sulfate magnésique entraîne à la dose de 150 à 200 mg. une modération marquée de l'excrétion pancréatique maintenue en activité par l'administration intraveineuse continue d'une solution diluée de sécrétine.

2° Cette même diminution sécrétoire s'observe lorsque l'on procède à deux stimulations répétées par injection d'une même dose de sécrétine pratiquées avant et dix minutes après l'administration de cette substance hypnotique.

3° Le pouvoir fermentaire du suc recueilli accuse une diminution de la teneur en lipase évaluée en totalisant la quantité d'enzymes émises au cours de l'épreuve.

4° L'inhibition sécrétoire pancréatique postmagnésienne ne se manifeste plus après section médullaire haute chez les chiens vagotomisés. Ce phénomène semble donc reconnaître une origine nerveuse.

Bibliographie

1. — La Barre, J. et Veselovsky, O. — Sur l'action du sulfate sodique sur les sécrétions de la salive et sur l'hypersécrétion gastrique pancréatique. *Arch. int. Pharmacodyn. et Ther.*, 1931, LXVI, 419-439.

2. — La Barre, J. et Veselovsky, O. — A propos de l'action de dérivés [illegible] sur la sécrétion gastrique et pancréatique. *Bull. de Thér. Méd. Belgique*, 1931, 6e s., VI, 180-180.

3. — Gaster, H. W. et Verhoeven, G. V. — The influence of magnesium sulfate on the secretory activity of the digestive glands. *Amer. Journ. Med. Sc.*, 1930, CLXXIX, 373-380.

4. — Maugeri, P. — Azione del solfato di magnesio sulla secrezione pancreatica esterna. *Boll. Soc. med. chir. Catania*, 1933, III, 390-392.

5. — Gröschel, H. — Über die Veränderung der Sekretionstätigkeit des Pankreas unter dem Einfluss von K, Ca und Mg. *Zeits. ges. exper. Med.*, 1928, I, 82-91.

Voprosy Onkologii 13 (8):121 1961

EFFECT OF MAGNESIUM SALTS ON INDUCTION BY URETHAN OF PULMONARY ADENOMAS IN MICE

B. M. Labkovskiy
Petrov Institute of Oncology

Stick-bred white mice mice weighing 19 to 20 g were used in the experiments. Urethan was injected intraperitoneally twice at 4-day intervals at 1 g/kg in a 5% solution. Magnesium salts were administered every day perorally or subcutaneously. When given perorally, the daily dose of the magnesium ion was 320 mg/kg; when injected subcutaneously, 10 mg/kg.

In series 1, the mice received $MgCl_2$ with drinking water for 10 weeks (started 2 weeks prior to the first injection of urethan). In the other series of experiments, magnesium administration was started 1 day prior to the first injection of urethan and it was continued for 10 days. In series 2, 3, and 4, the mice received $MgCl_2$ through a stomach tube; in another series they received $MgSO_4$. In series 6, the mice received subcutaneous injections of $MgSO_4$. Each series had its own control. The mice were sacrificed 3 months after the injection of urethan. The average number of pulmonary adenomas per control mouse was taken as 100%.

In the experimental groups, the average number of adenomas per mouse was 135% in series 1, 216% in series 2, 215% in series 3, 112% in series 4, 194% and 196% in series 5, and 173% in series 6.

The effect of magnesium was studied at various times before and after the carcinogen was injected. $MgSO_4$ was injected subcutaneously at 5 ml/kg of a 0.5% solution daily for 10 to 15 days.

Group 1 mice received urethan 2 days after the cessation of the magnesium injections. In group 2, magnesium treatment was initiated 10 days after the second injection of urethan. Group 3 received magnesium 2.5 months after the injection of urethan. Group 4 was the control.

If the average number of pulmonary adenomas per control mouse is 100%, it came to 174%, 146%, and 114%, respectively, in the experimental groups.

In our experiments magnesium stimulated the induction of pulmonary adenomas in mice by urethan. The method of injecting magnesium and substitution of SO_4 for Cl_2 had no significant effect on its action.

Voprosy Onkologii 13 (8) : 121 (1967)

ВОПРОСЫ ОНКОЛОГИИ, ТОМ XIII, № 8, 1967 г.

УДК 612.24-006.55-092.9-085.782-02 : 546.46

О ВЛИЯНИИ СОЛЕЙ МАГНИЯ НА ИНДУКЦИЮ УРЕТАНОМ АДЕНОМ ЛЕГКИХ У МЫШЕЙ

Б. М. Лабковский B. M. Labkovskii

Из лаборатории лекарственных методов профилактики и лечения злокачественных новообразований (зав.— засл. деят. науки проф. Н. В. Лазарев) Научно-исследовательского института онкологии им. проф. Н. Н. Петрова МЗ СССР (дир.— чл.-корр. АМН СССР проф. А. И. Раков)

В опытах были использованы белые беспородные мыши-самцы, весом 19—21 *г*. Уретан вводили внутрибрюшинно дважды с интервалом в 4 дня в дозе 1 *г/кг* в виде 5% раствора. Соли магния давали ежедневно через рот или подкожно. При даче через рот суточная доза иона магния составляла 320 *мг/кг*, при подкожном введении — 10 *мг/кг*.

В I серии опытов мыши получили $MgCl_2$ с питьевой водой в течение 10 недель (начало — за 2 недели до первой инъекции уретана). В остальных сериях введение магния начиналось за 1 день до первой инъекции уретана и продолжалось 10 дней. Во II, III и IV сериях мышам давали $MgCl_2$ через желудочный зонд, а другой — $MgSO_4$. В VI серии мышам производились подкожные инъекции $MgSO_4$. Каждая серия имела свою контрольную группу. Через 3 месяца после введения уретана мышей забивали. Среднее число аденом легких на 1 мышь в контроле принималось за 100%. The av. no. adens in lungs on 1 mouse in controls was 100%

В подопытных группах среднее число аденом на 1 мышь было: в I серии — 135%, во II—216%, в III—215%, в IV—112%, в V — 194% и 196%, в VI—173%.

Исследовалось влияние магния при введении его в различные сроки до или после инъекций канцерогена. $MgSO_4$ вводился подкожно по 5 *мл/кг* 0,5% раствора в день в течение 10—15 дней.

В 1-й группе мыши получали уретан через 2 дня после окончания инъекций магния, во 2-й группе начинали вводить магний через 10 дней после второй инъекции уретана, в 3-й группе — мышам вводили магний через 2,5 месяца после инъекции уретана, 4-я группа — контрольная.

Если среднее число аденом легких на 1 мышь в контроле принималось за 100%, то в подопытных группах оно составляло соответственно 174%, 146%, 114%.

В условиях наших опытов магний стимулировал индукцию уретаном аденом легких у мышей. Способ введения магния и замена аниона Cl^- на SO^{--} в его солях не оказывали существенного влияния на это действие.

THE CLINICAL AND BIOCHEMICAL EFFECTS OF EXCESS MAGNESIUM IN THE NEWBORN

Philip J. Lipsitz, M.B., M.R.C.P., D.C.H.

From the Department of Pediatrics, Beth Israel Medical Center and Mt. Sinai School of Medicine, New York

ABSTRACT. The effect of two regimens of magnesium sulfate therapy in the toxemic mother and her newborn is reviewed. When magnesium sulfate is given intramuscularly to the mother, the newborn is usually not compromised by excess magnesium but may be affected. If continuous intravenous infusion of magnesium sulfate is used and especially if given for more than 24 hours, one can anticipate a newborn manifesting all the signs of hypermagnesemia.

Serum levels in the cord blood and serial samples of the newborn do not necessarily correlate with the clinical picture and may be of little diagnostic value.

In contrast to the adult, the newborn does not excrete a magnesium load satisfactorily.

Magnesium toxicity should be recognized and treated as outlined in the infant of a toxemic mother who has received a large dose or prolonged magnesium sulfate therapy. *Pediatrics*, 47:501, 1971. MAGNESIUM TOXICITY, MANAGEMENT RENAL EXCRETION.

MAGNESIUM sulfate administered by the intravenous route or intramuscular route has been advocated in the treatment of toxemia of pregnancy. In 1967 we[1] reported 16 cases of hypermagnesemia in the newborn, and suggested that neonatal hypermagnesemia was deleterious to the newborn in that respiratory failure and hyporeflexia resulted. The opportunity has now presented itself to study the effect of magnesium sulfate dosage, route of administration, length of administration and infant size upon neonatal serum magnesium levels, the resultant morbidity and urinary excretion of magnesium in the newborn.

MATERIAL AND METHODS

Group I consists of 29 newborn infants delivered from 27 toxemic mothers ranging in age from 13 to 39 years. These pregnant mothers all received intravenous magnesium sulfate ($MgSO_4 \cdot 7\ H_2O$ USP) starting with an initial loading dose of 2 to 4 gm followed by continuous intravenous infusion at the rate of 1 gm/hour as outlined by Zuspan.[2] The dose of magnesium sulfate administered was from 5 gm in 2½ hours to 90 gm in 83 hours prior to delivery. The period of gestation ranged from 34 to 42 weeks, and the birth weights of the newborns varied from 970 to 4,370 gm.

Group II consists of eight newborn infants of 8 toxemic mothers ranging in age from 19 to 31 years. Magnesium sulfate was given mainly intramuscularly after an initial intravenous dose of 2 to 3 gm. The total dose of magnesium sulfate was from 7 gm in 4 hours to 53 gm in 23 hours. The period of gestation varied from 33 to 40 weeks, and the birth weight of the newborn infants varied from 907 to 3,544 gm.

At birth, simultaneous maternal venous and cord blood samples (mixed venous and arterial) were obtained for magnesium, calcium, and total proteins determination. Serial heel-stick blood samples at approximately 12 hour intervals were obtained from the newborn infants, and urine was collected when possible.

Magnesium was determined in duplicate by three different methods. Originally the method of Bouhuon[3] using Mann's Dye was employed; later the rapid ultramicrospectrophotometric determination of Rice and Lapara[4] was used. When an Atomic Absorption Spectrophotometer Perkin Elmer

(Received June 5; revision accepted for publication September 16, 1970.)

ADDRESS FOR REPRINTS: Beth Israel Medical Center, 10 Nathan D. Perlman Place, New York, New York 10003.

TABLE I

CLINICAL AND BIOCHEMICAL EFFECTS OF $MgSO_4$ ADMINISTRATION IN THE MOTHER AND HER NEWBORN

Patient	Age in Years	Gestation in Weeks	Dose of $MgSO_4$ in grams and Route		Duration of Administration Prior to Delivery (Hours)	Maternal Serum at Delivery: Mg^{++} mEq/l	Maternal Serum at Delivery: Ca^{++} mEq/l	Maternal Serum at Delivery: Protein Gm/100 ml	Newborn: Birth weight (Gm)	Newborn: Apgar at 1 Minute	Newborn: Clinical Score	Newborn Cord Serum: Mg^{++} mEq/l	Newborn Cord Serum: Ca^{++} mEq/l	Newborn Cord Serum: Protein Gm/100 ml	Outcome	Resuscitation and or Assisted Ventilation
Group I																
1*	22	28	27	IV	8	5.5	4.0	5.3	970	3	3	6.0	5.3	4.6	D	R,V
2*	18	30	70	IV	72	4.5	3.0	ND	1,204	2	3	4.7	4.0	ND	D	R,V
3*	30	36	50	IV	38	ND	ND	ND	1,286	1	3	4.7	ND	5.6	L	R
4*	22	30	25	IV	11	3.2	3.4	6.3	1,292	4	3	3.2	4.4	5.6	D	R,V
5*	45	32	41	IV	38	5.4	ND	ND	1,431	4	3	5.6	ND	ND	D	R,V
									1,500	5	3	ND	ND	ND	D	R,V
6*	35	35	42	IV	36	6.1	4.5	6.9	1,690	1	3	6.3	4.6	5.9	L	R,V
7	18	40	30	IV	19	5.2	3.9	8.0	1,705	2	3	4.7	3.5	5.9	L	R
8*	34	40	16	IV	20	3.0	3.6	6.6	1,774	8	0	3.1	4.0	5.0	L	
									2,011	8	0	2.8	ND	4.6	L	
9*	17	32	37	IV	35	3.0	3.5	6.0	1,780	4	2	3.4	4.2	6.6	L	
10	14	35	17	IV	8	4.6	3.2	6.1	1,835	9	0	4.5	4.2	6.4	L	
11	15	36	30	IV	16	2.8	3.6	5.5	2,205	1	3	4.6	3.8	6.4	L	R
12*	16	40	30	IV	13	2.6	3.1	7.3	2,225	9	0	3.0	4.3	8.0	L	
13	19	36	5	IV	2.5	6.4	4.3	7.9	2,500	5	1	6.1	4.6	7.1	L	
14	39	38	25	IV	18.5	6.6	4.1	8.6	2,520	6	1	6.0	5.0	8.4	L	
15*	17	40	60	IV	20	8.0	ND	ND	2,555	9	0	6.6	ND	ND	L	
16*	17	36	42	IV	27.5	3.1	3.1	5.9	2,630	5	2	3.0	4.2	6.1	L	
17	23	38	5	IV	5	ND	ND	ND	2,650	8	1	3.4	5.2	6.1	L	
18	18	40	26	IV	20	6.1	3.7	7.7	2,780	5	2	5.3	4.2	7.0	L	
19	15	40	46	IV	25	3.1	ND	7.3	2,840	6	1	4.0	4.6	8.2	L	
20*	17	40	56	IV	33	14.0	ND	8.4	2,860	2	3	11.5	5.2	6.4	L	R,V
21*	19	40	17	IV	4	3.5	3.4	5.8	2,900	5	2	3.7	5.2	6.1	L	
22*	15	40	25	IV	21	ND	ND	ND	3,105	4	2	4.4	5.0	5.7	L	R
23	39	38	16	IV	20	2.9	4.1	6.2	3,175	2	3	3.1	4.3	7.3	L	R
24*	22	40	90	IV	88	6.3	ND	5.3	3,401	7	1	7.4	4.1	5.9	L	
25*	18	40	76	IV	72	6.4	3.4	4.6	3,711	1	3	5.9	4.3	5.3	L	R
26	19	39	35	IV	36	2.9	3.7	5.4	3,809	2	3	3.3	3.7	7.0	L	R
27*	18	40	19	IV	11	4.6	4.2	6.1	4,370	6	2	3.4	5.5	6.9	L	
Group II																
1	21	34	4	IV	2	3.7	4.1	ND	907	1	3	4.7	4.6	ND	L	R
			10	IM												
2*	31	35	3	IV	23	6.9	4.0	ND	2,098	1	3	3.4[N]	4.2[N]	ND	L	R,V
			50	IM												
3	19	37	3	IV	2.5	4.8	3.7	6.7	2,807	8	0	4.4	4.7	ND	L	
			10	IM												
4	26	38	12	IV	6	4.0	4.7	ND	2,892	9	1	3.5	5.0	ND	L	
5	26	40	3	IV	9	5.8	4.5	6.9	3,252	3	3	3.5	4.6	ND	L	R
			15	IM												
6	19	40	7	IV	4	2.7	4.8	ND	3,459	7	0	3.3	5.3	ND	L	
7	25	40	4	IV	8	3.1	4.8	ND	3,515	7	0	3.4	ND	ND	L	
			15	IM												
8	27	39	3	IV	3	4.5	ND	ND	3,544	9	0	3.5	5.0	ND	L	
			10	IM												

* Eclamptic.
ND = Not determined.
L = Living.
D = Died.
N = At 20 minutes of age.
R = Resuscitation.
V = Assisted Ventilation.

Model 2903[6] became available this was used. The three methods were evaluated by recovery experiments and a good correlation (95% confidence) was found among all three methods.

Serum was frozen and calcium was determined in duplicate by the Atomic Absorption Spectrophotometer in one run. Total protein in duplicate was determined by the modified Biuret method of Kingsley.[5]

RESULTS

Table I demonstrates the biochemical data and outcome of all patients studied in Groups I and II. Five newborn infants in Group I died; all were less than 1,500 gm at birth and less than 32 weeks' gestation. None of the Group II newborn infants died. Two newborn infants in Group I and one in Group II, who required assisted ventilation, survived.

Column 3 under Newborn in Table I shows the clinical (hypermagnesemic) score of each infant, which is an arbitrary score for assessing symptomatology related to magnesium toxicity. At 1 to 60 minutes after birth, one point is given for each sign: (1) flaccidity and hyporeflexia; (2) need for resuscitation or assisted ventilation; and (3) a weak or absent cry unrelated to tracheal intubation. The higher the score, with a maximum of 3, the greater the *apparent* toxicity of excess magnesium.

The serum magnesium levels of paired maternal and cord blood, in normal uncomplicated pregnancies, determined by the methods outlined are illustrated in Figure 1. The mean normal maternal serum magnesium level was 1.8 mEq/l (1.2 to 2.2), S. D. 0.3, and cord blood serum magnesium level 1.9 mEq/l (0.9 to 2.6) S. D. 0.3. This figure also illustrates a scattergram of the maternal and cord serum magnesium levels in Group I. After infusion of 5 to 90 gm magnesium sulfate, the mean maternal level for magnesium was 5.0 mEq/l (2.6 to 14.0) S. D. 2.4 in 24 patients, and the mean cord magnesium level was 4.8 mEq/l (2.8 to 11.4) S. D. 1.84 in 28 samples. Group II presented with a mean maternal serum magnesium level of 4.2 mEq/l (2.7 to 6.9) S. D. 1.20 and mean cord serum magnesium level of 3.7 mEq/l (3.3 to 4.7) S. D. 0.49 after administration of 7 to 53 gm of magnesium sulfate.

The protein and calcium determinations in the maternal and cord blood are illustrated in Table I. In Group I the serum calcium in maternal blood had a low mean value of 3.7 mEq/l (3.0 to 4.5) S. D. 0.46. Cord blood serum calcium levels were higher with a mean of 4.5 mEq/l (3.5 to 5.3) S. D. 0.54. Group II presented with mean serum calcium levels of 4.4 mEq/l (3.7 to 4.8) S. D. 0.41 and 4.8 mEq/l (4.2 to 5.3) S. D. 0.33 respectively in maternal and cord blood.

The serum magnesium and calcium levels were followed serially in some of the newborn infants (Table II). This table indicates how slowly the serum magnesium level decreased in the newborn, and that some newborn infants did not have normal serum levels till 60 to 80 hours of age even though they were asymptomatic. Some babies did manifest serum calcium values as low as 3.3 mEq/l over the period of sampling. None of these hypocalcemic levels was associated with signs of tetany. Low serum calcium levels have been observed in premature infants and may also be related to the parental fluid therapy devoid of calcium administered to the newborn. In Group II the serum calcium levels were all greater than 4 mEq/l at the times of sampling.

The total serum proteins did not show any definite trend, and clinical manifestations of hypermagnesemia in the newborn could not be related to low serum protein levels.

In adults, infused magnesium is excreted in the urine; it was hoped that a similar experience would be found in the newborn. Table III illustrates the initial cord serum magnesium level, urinary volume, and uri-

[6] Perkin Elmer, Norwalk, Connecticut.

TABLE II

MATERNAL, CORD AND SERIAL SERUM Mg^{++} AND Ca^{++} IN THE NEWBORN

Patient	Maternal Serum at Delivery		Cord Blood		Serial Newborn Serum					
					0–12 hrs.		12–24 hrs.		24–48 hrs.	
	Mg^{++} mEq/l	Ca^{++} mEq/l	Mg^{++} mEq/l	Ca^{++} mEq/l	Mg^{++} mEq/l	Ca^{++} mEq/l	Mg^{++} mEq/l	Ca^{++} mEq/l	Mg^{++} mEq/l	Ca^{++} mEq/l
Group I										
4	3.2	3.4	3.2	4.4	3.0	4.3	Expired			
5	5.4	ND	5.6	ND	5.4	ND	Expired			
					4.4	3.3	4.3	ND	Expired	
6	6.1	4.5	6.3	4.6	ND	ND	5.0	3.6	4.1	3.3
7	5.2	3.9	4.7	3.5	ND	ND	4.0	ND	ND	ND
13	6.4	4.3	6.4	4.6	3.9	ND	3.3	ND	ND	ND
15	8.0	ND	6.6	ND	ND	ND	4.3	ND	ND	ND
18	6.1	3.7	5.3	4.2	ND	ND	4.2	3.7	ND	ND
20	14.0	ND	11.5	5.2	8.1	3.7	6.2	ND	4.2	3.7*
21	3.5	3.4	3.7	5.2	3.2	5.7	2.4	5.3	ND	ND
24	6.3	ND	7.4	4.1	5.3	ND	ND	ND	ND	ND
25	6.4	3.4	5.9	4.3	5.1	ND	4.0	ND	4.2	ND*
26	2.9	3.7	3.3	3.7	ND	ND	2.5	4.0	ND	ND
27	4.6	4.2	3.4	5.5	2.6	4.6	2.4	4.0	ND	ND
Group II										
2	6.9	4.0	3.4	4.2	3.6	3.6	4.3	4.4	3.0	4.2*
3	4.8	3.7	4.4	4.7	2.9	4.7	2.9	4.6	2.6	4.4
4	4.0	4.7	3.5	5.0	3.1	4.9	1.6	4.9	2.8	4.6
5	3.8	4.5	3.5	4.6	3.4	4.7	2.9	4.6	2.6	4.4
6	2.7	4.8	3.3	5.3	ND	5.1	3.1	4.8	2.2	ND
7	3.1	4.8	3.4	ND	ND	ND	2.8	4.1	2.8	4.6

* Serum magnesium elevated 60–80 hours of age.
ND = Not determined.

nary magnesium excretion in seven infants in both groups. The amount of magnesium excreted is usually less than 1 mEq in each time period of urine collection, indicating a very low excretion rate.

Statistical analysis of the present data of 29 Group I newborn infants, by Pearson's correlation coefficient, shows no correlation between:

(1) Apgar score at 1 minute and birth weight, cord serum magnesium level, and total dose of magnesium sulfate prior to delivery; (2) serum magnesium levels in maternal and cord blood and, total dose of magnesium sulfate administration prior to delivery; (3) magnesium toxicity score and total does of magnesium sulfate administration prior to delivery; (4) birth weight and cord serum magnesium level.

DISCUSSION

Studies have shown that magnesium administered to the mother will cross the placenta to the fetus. Pritchard,[9] in a report on the use of magnesium sulfate in the management of toxemia of pregnancy, found a delay of 2 hours in establishing equilibrium between maternal and fetal plasma. His study also indicated that the injected magnesium distributed beyond the extracellular space.

Aikawa and Burns[7] studied the placental transfer and fetal tissue uptake of Mg^{28} in the rabbit. After a single intravenous injection of Mg^{28} there was a rapid fall in the isotope concentration of maternal plasma during the first 2 hours. The isotope concentration in the placenta was initially lower than in the maternal plasma, but

within 2 hours the placental concentration reached higher levels and remained so. Concentration of Mg^{28} in the fetus was initially low but increased gradually, and at 24 hours exceeded both maternal and placental concentration of the isotope. Relative magnesium concentration in fetal tissues remained consistent in the following order: bone (highest), muscle, liver and kidney (intermediate), and lung (lowest). Specific activities of all fetal tissues approached a constant value at 24 hours suggesting that Mg^{28} was at equilibrium with the untagged element in these tissues.

The average 70 kg-adult has about 2,000 mEq of magnesium in his body and about half the body store of magnesium is present in the intracellular compartment and half is present in bone. About 70 to 80% of serum magnesium is ionized and diffusible, the remainder being probably bound to protein. The serum level remains constant at about 2.0 mEq/l. It is, thus, reasonable to assume that the serum magnesium (extracellular compartment) does not reflect the total body magnesium content. Widdowson and McCance[6] indicate that a newborn weighing 3.5 kg will have about 600 mEq of mag-

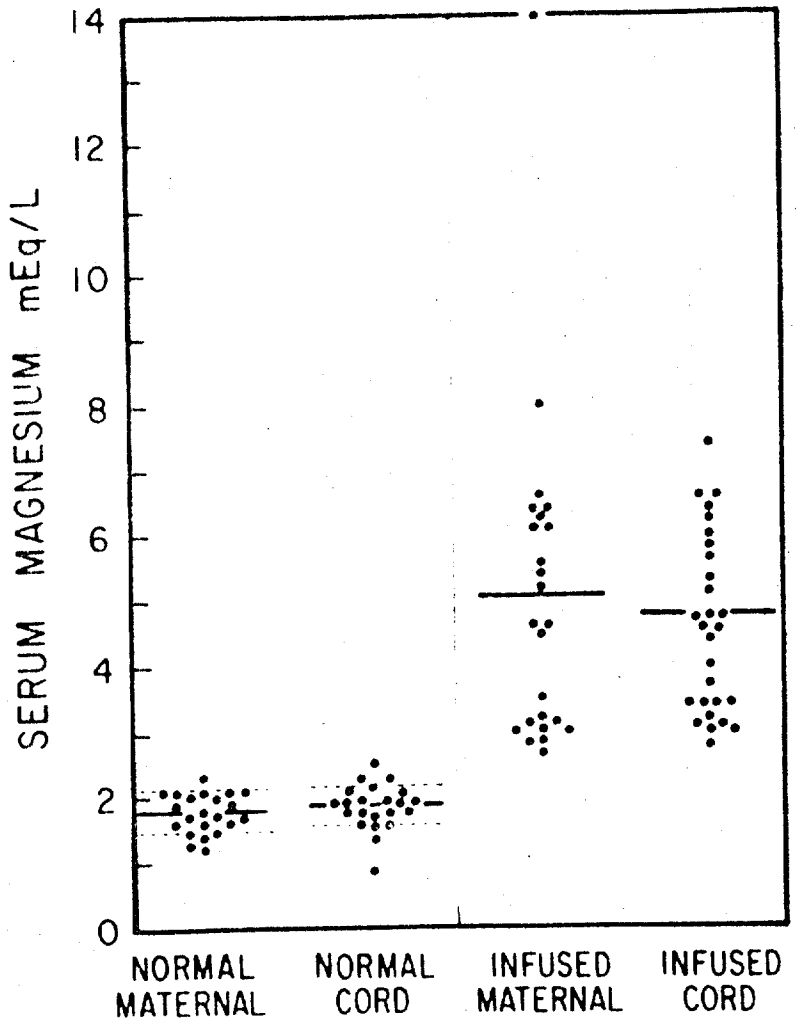


FIG. 1. Serum magnesium levels in paired maternal and cord blood in uncomplicated pregnancies and scattergram of maternal and cord blood serum in Group I babies.

TABLE III

URINE EXCRETION OF MAGNESIUM

Patient	Cord Blood Mg ++ mEq/l	Age of Newborn in Hours	Urine Volume (ml)	Urinary Mg ++ mEq/l
I 6	6.3	0-24	45	26.8
I 25	5.9	12-36	50	35.0
I 26	3.3	0-24	44	20.3
		24-48	250	0
II 2	3.4	12-24	110	5.4
		24-48	140	5.4
II 4	3.5	12-24	20	4.9
		24-48	23	2.3
II 5	3.5	0-12	10	6.2
II 6	3.3	0-12	15	0.8

nesium in his body. The data of Aikawa and Bruns[8] suggest that in pregnancy, after a single intravenous dose of magnesium, the fetal tissues approach a constant value at 24 hours. The more frequent and repetitive administration of magnesium prior to delivery of the baby could be anticipated to give rise to a higher intracellular concentration in the newborn. In addition, a continuous intravenous infusion of magnesium could be expected to concentrate even more magnesium in the tissues. The discrepancy between serum magnesium values and clinical symptomatology would suggest that the total body content or intracellular magnesium content is critical in determining the clinical manifestations, or else there is an interplay between several related ions and pH, both intra- and extracellularly which determines the neuromuscular activity.

Stone and Pritchard[9] could not demonstrate any deleterious effect of maternal intramuscular magnesium administration on the newborn, nor could they relate the serum magnesium level in the newborn to the Apgar score or neonatal mortality.

The present study confirms the opinions expressed by Stone and Pritchard,[9] but does not necessarily imply that other factors contribute to the clinical picture noted in Group I. We commented previously[1] that the clinical manifestations of hypermagnesemia in the newborn were compatible with the findings of Randall, *et al.*[10] in adults with hypermagnesemia, and unlike those of newborn infants of toxemic mothers without magnesium sulfate therapy.

However, one notes from Figure 2, that in up to 24 hours of continuous intravenous infusion of magnesium sulfate, 13 of 17 newborn infants had Apgar scores at 1 minute of 4 or higher. After 24 hours of infusion only 6 or 12 newborn infants had Apgar scores of 4 or higher. The continuous infusion of magnesium sulfate for more than 24 hours prior to delivery indicates a trend to score low on the Apgar score at 1 minute, but is not statistically significant ($p > 0.3$).† Furthermore, Figure 3 illustrates that up to 24 hours of continuous intravenous infusion of magnesium sulfate, 9 of 17 newborn infants scored 2 or higher on the proposed clinical score of hypermagnesemia, and after 24 hours of infusion, 12 of 12 scored 2 or higher. This is statistically significant ($0.02 > p > 0.01$),† and indicates that the longer the period of magnesium administration prior to delivery, the more manifestations of excess magnesium will appear in the newborn. The data in Table I indicates how difficult it is to correlate the clinical manifestations with high serum magnesium levels, especially in patients 10, 13, and 15 of Group I.

The number of patients in Group II is too small for statistical analysis but, again, illustrates the difficulty of relating the clinical manifestations with the magnesium levels. Only three of eight newborn infants had low Apgar scores at 1 minute and high magnesium toxicity scores. This group seems to confirm the impression that intramuscular administration to the mother is not deleterious to her newborn, but Patient 2 would suggest that even repeated intra-

† Chi-square.

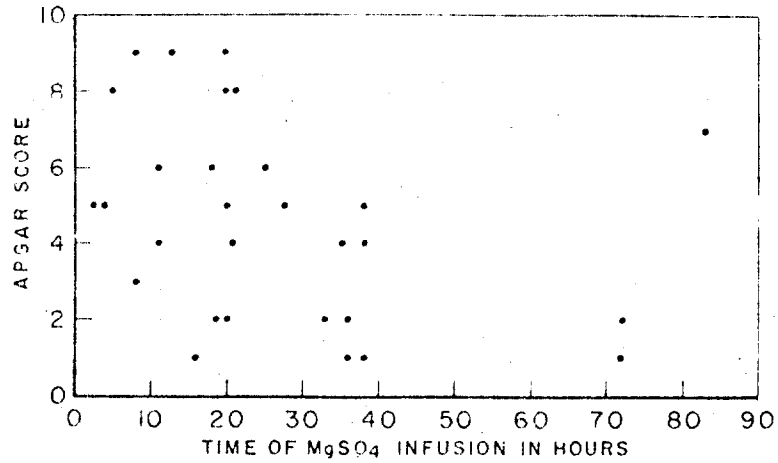


FIG. 2. The relationship of the Apgar Score to the duration of magnesium sulfate infusion in hours prior to delivery.

muscular administration to the mother may be deleterious to her newborn. At present no data is available as to the effect of repeated intramuscular administration over a period greater than 24 hours.

It may or may not be that the extracellular magnesium concentration is important in the clinical manifestation of hypermagnesemia. After prolonged infusion (24 hours or more of magnesium sulfate in the mother) one would expect the fetal tissue concentration to be higher, and as noted after 24 hours, more newborn infants were symptomatic as expressed by the clinical score. This would explain why, as noted previously,[1] some newborn infants did not have normal magnesium levels until after 80 hours of age (Table II). Serum values were not followed further in all infants as they were clinically normal.

The clinical manifestations in the newborn could be related to severe asphyxia, but this would not explain those cases with high clinical scores and Apgar scores of 5 or greater. Central nervous system hemorrhage can present a similar clinical picture in the newborn, but convulsions and localizing neurological deficits would be more common, and so complete recovery in 24 to 48 hours[1] without evidence of any neurological deficit would not be expected.

Calcium and magnesium homeostasis are

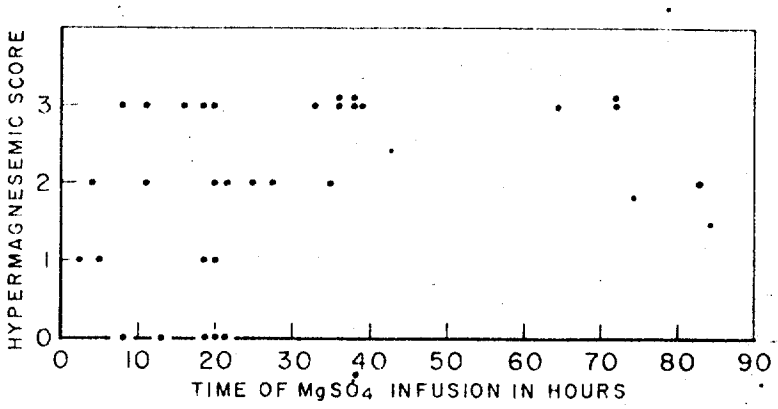


FIG. 3. The clinical score (hypermagnesemic score) in relationship to the duration of the magnesium sulfate infusion in hours prior to delivery.

interdependent. MacIntyre, *et al.*[7] have published evidence that a rise in serum magnesium will inhibit secretion of parathyroid hormone resulting in increased urinary excretion of calcium. From the maternal serum calcium levels in Group I at the time of delivery when serum magnesium levels are elevated, one notes that the mean serum calcium was 3.7 mEq/l (3.0 to 4.5) in 19 patients, the mean cord serum calcium level being 4.5 mEq/l (3.5 to 5.5). The low maternal calcium may be related to hypercalciuria following magnesium sulfate administration over several hours. When magnesium sulfate was administered mostly intramuscularly (Group II) and the duration of therapy was shorter, the mean maternal serum calcium was 4.4 mEq/l (3.7 to 4.8) and the cord serum calcium was 4.8 mEq/l (4.2 to 5.3).

MANAGEMENT OF THE NEWBORN

How the newborn, who is compromised from excess magnesium, should be handled has not been fully evaluated.

The newborn may require resuscitation and assisted ventilation (Table I) until the depression of his respiratory center and muscle weakness is overcome. This is best achieved by endotracheal intubation and intermittent positive pressure ventilation. Blood gases and acid-base balance will help in the management of the infant. When the newborn shows spontaneous respiratory efforts, which may be achieved in 6 to 48 hours, he can be taken off the respirator.

Calcium has been used to antagonize the central nervous system depression and peripheral transmission defect of excess magnesium. Two mothers in Group I received calcium, 1 gm intravenously, prior to delivery of their neonates, but both newborn infants were flaccid at delivery and one newborn in addition required resuscitation. Five of the newborn infants in this series received from 200 to 500 mg calcium intravenously diluted in 10 ml of saline, given slowly with monitoring of the heart rate. In only one infant was there an apparent improvement following this therapy. Brady[12] has had similar experience with intravenous calcium given to the compromised hypermagnesemic newborn. From the evidence already presented, it seems unlikely that calcium will antagonize all the magnesium which is stored in the muscle and bone. Thus, calcium may temporarily improve the clinical hypotonia, but would not be expected to reverse completely the picture in the newborn.

Intravenous administration of fluid usually at a rate of 65 ml/kg/day of 10% dextrose in water for the first 24 hours, followed by intravenous fluids at a rate of 85 ml/kg/24 hours of 10% dextrose in water with 0.2% saline is used to maintain electrolyte balance in the newborn and to force a diuresis in the infant. The results of the urinary excretion of magnesium, Table III, would suggest that forced diuresis does not appear to be a satisfactory method of managing these infants, but intravenous therapy is indicated for fluid and electrolyte balance and for the correction of metabolic acidosis.

Brady and Williams[13] published one case report of reversal of magnesium toxicity in a newborn by exchange transfusion. The clinical and biochemical manifestation improved dramatically during the procedure. It may be that this is the method of choice in the management of the newborn severely compromised due to excess magnesium. However, it is still questionable whether sufficient magnesium will be removed from the tissues during the procedure so that the clinical manifestations will disappear after exchange transfusion.

It appears thus far that with conservative management by maintaining ventilation and fluid and electrolyte balance, there is a marked improvement in the clinical condition of the newborn at 24 to 36 hours of age. When the newborn manifests, at that age usually with a good suck reflex, oral feeding can be started, but some may still require intravenous fluids or even assisted respiration.

REFERENCES

1. Lipsitz, P. J., and English, I. C.: Hypermagnesemia in the newborn infant. PEDIATRICS, 40:856, 1967.
2. Zuspan, F. P.: Treatment of severe preeclampsia and eclampsia. Clin. Obstet. Gynec., 9: 954, 1966.
3. Bolmon, C.: Microdosage du magnesium dans divers milieux biologiques. Clin. Chim. Acta., 7:811, 1962.
4. Rice, E. W., and Lopyra, C. Z.: Rapid ultramicrospectrophotometric determination of magnesium. Clin. Chim. Acta., 10:360, 1964.
5. Kingsley, G. R.: The determination of serum total protein, albumin and globulin by the biuret reaction. J. Biol. Chem., 131:197, 1939.
6. Pritchard, J. A.: The use of the magnesium ion in the management of eclamptogenic toxemias. Surg. Gynec. Obstet., 100:131, 1955.
7. Aikawa, J. K., and Bruns, P. D. Placental transfer and fetal tissue uptake of Mg^{28} in the rabbit. Proc. Soc. Exper. Biol. Med., 105: 95, 1960.
8. Widdowson, E. M., and McCance, R. A.: The metabolism of calcium, phosphorus, magnesium and strontium. Pediat. Clin. N. Amer., 12:595, 1965.
9. Stone, S. R., and Pritchard, J. A.: Effect of maternally administered magnesium sulfate on the neonate. Obstet. Gynec., 35:574, 1970.
10. Randall, R. E., Jr., Cohen, M. D., Spray, C. C., Jr., and Rossmeisl, E. C.: Hypermagnesemia in renal failure—etiology and toxic manifestations. Ann. Intern. Med., 61:73, 1964.
11. MacIntyre, I., Boss, S., and Troughton, V. A.: Parathyroid hormone and magnesium homeostasis. Nature, 198:1058, 1963.
12. Brady, J. P.: Personal communication.
13. Brady, J. P., and Williams, H. C.: Magnesium intoxication in a premature infant. PEDIATRICS, 40:100, 1967.

Acknowledgment

The author is indebted to the Department of Obstetrics and Gynecology at the Medical College of Georgia, and the Department of Obstetrics and Gynecology at the Beth Israel Medical Center, New York, for their cooperation in the study.

Pediatrics 40: 856-62

HYPERMAGNESEMIA IN THE NEWBORN INFANT

Philip J. Lipsitz, M.B. (Capetown), M.R.C.P. (Ed.), D.C.H. (London) and
Inman C. English, M.D

From the Department of Pediatrics, Medical College of Georgia, Augusta, Georgia

ONLY ONE case report of hypermagnesemia in a newborn has been published. Hutchinson, *et al.*,[2] in a report on effects of magnesium sulfate on uterine contractility, intra-uterine fetus, and infant, found oscillation in the fetal heart rate, after an injection of 4 gm $MgSo_4$, inconsistent and short lasting. The direct effect of Mg on the fetal circulation could not be excluded. In their report of one infant, in whom maternal serum magnesium level was 4.5 mEq/l and fetal serum magnesium immediately post delivery was 3.8 mEq/l, the infant was normal at birth with good muscle tone, pink color, and heart rate of 130 and responded instantly to nasal suction by breathing and crying. They concluded, in their study of 34 women who received magnesium during labor (ranging from a single dose of 4 gm to as much as 16 gm during 10 hours), that no evidence of adverse action of magnesium on the infant was observed. Since magnesium sulfate is often given, in the management of toxemia of pregnancy,[3] either intramuscularly or by intravenous infusion, it was decided to study the effect of such maternal administration of magnesium sulfate upon the newborn infant.

The direct effect of toxemia on the fetus produces a high perinatal mortality with an increase in still births and neonatal deaths. An analysis of the outcome of eclamptic pregnancies at the teaching hospital of the Medical College of Georgia reflects this fact (Fig. 1) and that, since 1963–1965, the neonatal death rate has apparently decreased, perhaps as a result of the increasing use of larger doses of maternal intravenous magnesium sulfate prior to delivery.

MATERIAL AND METHODS

The obstetrical department of the Medical College of Georgia has proposed the use of magnesium sulfate only in the treatment of severe preeclampsia and eclampsia. This afforded us an opportunity to study the effect of such management on the newborns.

With the cooperation of the obstetric department, maternal serum was obtained at the time, or as near as possible to the time of delivery from toxemic mothers infused with magnesium sulfate. Blood was allowed to drip into sterile tubes for the cord blood samples after delivery. Blood was allowed to clot and then centrifuged at 2,000 rpm for 10 minutes. The supernatant sera were removed and frozen until analysis.

Magnesium was determined by the method of Bohuon[4] using Mann's Dye. The values obtained for normal paired maternal and cord serum magnesium levels in our laboratory are shown in Table I.

These values are similar to other reported values using different methods for the determination of magnesium in the newborn.[5-7]

From April 1965 to September 1966 paired samples were obtained from 11 of 17 cases of severe preeclamptic and eclamptic pregnant mothers infused with $MgSo_4$. This does not include any patient who received a single dose of $MgSo_4$ for mild preeclampsia. Because of the serious condition of the mother, maternal blood was not obtained prior to delivery in a twelfth case, but cord blood was obtained. In the five other patients maternal and cord samples were not obtained. In this group of 17 there was no maternal death, and only one stillborn and one neonatal death. Since this pe-

(Received March 16; revision accepted for publication June 30, 1967.)
Supported in part by a General Research Support Grant FR 05365.
ADDRESS: (P.J.L.) Department of Pediatrics, Eugene Talmadge Memorial Hospital, Augusta, Georgia 30902.

riod two other eclamptic pregnant mothers who were managed on a similar regimen of $MgSo_4$ delivered three newborns in this institution. The set of twins expired at 9 and 30 hours of age, respectively. The third neonate survived.

The infants on whom serial venous blood samples were obtained for magnesium determination were not necessarily those who manifested the most severe clinical course.

CASE REPORTS

The following detailed case reports indicate the clinical and biochemical changes associated with hypermagnesemia in newborns.

Case 1

A 17-year-old, Negro primipara was admitted on June 20, 1966, at 4:00 P.M. with an unknown LMP and estimated gestation by history of ± 40 weeks. She was seen in her local health clinic and treated with diuretics for preeclampsia 1 week prior to admission. On June 20, 1966, she had two grand mal seizures followed by unconsciousness. She was given sodium amytal and transferred to our institution from the local health clinic.

On admission at 4:00 P.M. she was still having seizures. Physical examination revealed the following: semicomatose and not in active labor; blood pressure, 152/104; pulse, 140; fetal heart rate, 156; estimated fetal weight, 7½ to 8 lb; edema, 2+; reflexes hyperactive. Laboratory values: urine 4+ proteinuria; hemogram normal; electrolytes normal.

She was immediately given 4 gm $MgSo_4$ intravenously, and a continuous infusion of 5% dextrose and water was commenced at 4:30 P.M. $MgSo_4$ was added to the intravenous fluids and a total of 51.5 gm was given until 12:53 A.M. on June 22, 1966, a period of 33.5 hours. The blood pressure decreased to 110/80, and the urinary output varied between 30 to 70 ml/hour. Reflexes during this period were all normal. A medical induction was started on June 21, 1966.

A live, female infant (birth weight, 2,820 gm; Apgar, 2 at 1 minute) was delivered at 12:53 A.M., June 22, 1966. Simultaneous maternal and cord serum was obtained. The placenta weighed 540 gm. Because of clinical amnionitis, the mother received penicillin and streptomycin postpartum.

The infant was foul smelling and required endotracheal intubation to establish respiration. The heart rate was constantly greater than 100.

Assisted ventilation for 2 hours was required by the infant who remained flaccid and unresponsive to stimuli. A chest roentgenogram was consistent with aspiration pneumonia.

A scalp vein infusion of 65 ml/kg/24 hours of 10% dextrose and water was started on the infant at the age of 3 hours and she also received 150 mg/kg/24 hours of ampicillin, divided in 12, hourly, intravenous doses. Ten milliliters of 10% calcium gluconate and 4.5 mEq of $NaHCO_3$ were also added to the intravenous fluids at the age of 9 hours.

The clinical course and biochemical changes of the neonate are described in Table II. Maternal serum magnesium was greater than 14 mEq/l.

She continued to do well and was discharged on the 15th hospital day.

Case 2

The mother was a 19-year-old Negro primipara with severe preeclampsia admitted prior to delivery at 36 weeks gestation. She received 21 gm $MgSo_4$ for 24 hours prior to delivery by continuous intravenous infusion in 5% dextrose and water.

A male infant was born at 9:00 A.M. on June 23, 1966. Apgar score was 5 at 1 minute and 10 at 5 minutes after birth. Birth weight was 2,500 gm. The heart rate was below 100 and the infant had minimal activity. The infant breathed spontaneously after nasopharyngeal suction but was noted to have a weak cry.

Maternal blood and cord blood was obtained simultaneously at delivery revealing a magnesium concentration of 5.3 mEq/l in both.

The infant was managed in the regular nursery and showed no further manifestation of hypermagnesemia. He voided soon after birth. The clinical and biochemical changes observed are shown in Table III.

The infant was discharged at 3 days of age with no further clinical evidence of hypermagnesemia or toxicity.

Case 3

The mother was an 18-year-old Negro primipara with a gestation of 41 weeks and severe pre-

TABLE I

VALUE FOR NORMAL SERUM MAGNESIUM LEVELS

	Maternal Mg mEq/l	*Cord Mg* mEq/l
Mean	1.77	1.86
N	22	22
S.E.M.	0.06	0.08
Range	1.15–2.30	0.90–2.55
P	Not significant	

TABLE II

CLINICAL COURSE OF INFANT, CASE 1

Age hr	Clinically	Acid-Base Balance	Mg^{++} mEq/l	Treatment
Birth	Depressed Apgar 2		11.5 (cord blood)	Intubated O_2 I.V. fluids
2	Lethargic; unresponsive; regular, shallow respiration	—	—	↑
8	Respiratory arrest	—	—	Intubated
9	Chest retraction; irregular respiration; voided	Respiratory and metabolic acidosis	8.1 (Calcium 7.6 mg/100 ml) (Phosphatase 9.6 mg/100 ml)	+Calcium gluconate+$NaHCO_3$
14	Spontaneous movement	—	—	
15	Moving; listless	Respiratory acidosis	6.2	
30	Poor muscle tone, crying	Respiratory acidosis	4.7	
36	Active; intercostal retraction	—	—	↓
54	More active; opening eyes	Normal	3.9	I.V. fluids
60	Accepting oral feeds	—	—	—
80	Normal activity	—	(Calcium 7.4 mg/100 ml)	

eclampsia. Membranes ruptured 20 hours before delivery. She received a total of 30 gm $MgSO_4$ by continuous intravenous infusion in 5% dextrose and water over 19 hours.

A male infant was delivered at 1:20 P.M. on August 19, 1966. Birth weight was 2,780 gm; Apgar was 5 at 1 minute and 10 at 5 minutes after birth. The infant was noted to be hypotonic and hyporeflexic for 5 to 6 minutes after delivery; his condition soon improved and he was transferred to the regular newborn nursery where he had an uneventful course and at 1 hour of age was responding normally to stimuli. Maternal magnesium level was 6.1 mEq/l, cord magnesium was 5.3 mEq/l, serum magnesium at age 21 hours was 4.2 mEq/l, and serum calcium was 9.7 mg/100 ml. He was discharged at the age of 10 days with no further complications.

Case 4

The mother was a 13-year-old primipara with severe preeclampsia admitted at term. She received 31 gm $MgSO_4$ intravenously over a 20-hour period by continuous infusion in 5% dextrose and water.

The infant, a male, had an Apgar score of 2 at 1 minute and required intubation and assisted respiration. The infant was floppy and had intercostal retraction. Birth weight was 1,980 gm. At age 4 hours he had poor, shallow respiratory movements but was not cyanotic. At 12 hours of age he was noted to have poor activity and responded sluggishly to stimuli with a weak cry.

Maternal magnesium was 5.2 mEq/l, cord magnesium was 4.7 mEq/l, and 12-hour serum magnesium was 4.0 mEq/l.

He continued to improve clinically. From 12

TABLE III

CLINICAL COURSE OF INFANT, CASE 2

Age hr	Clinically	Acid-Base Balance	Mg^{++} mEq/l	Treatment
Birth	Apgar 5 at 1 minute; 10 at 5 minutes; weak cry		5.3 (cord blood)	Nasopharyngeal suction
3	Voided; normal activity; respiratory rate 40/min		3.2	Observation
6	Active movements; respiration 40/min	Respiratory acidosis	2.5	Oral dextrose and water then formula
24	Normal activity	Normal	2.7	—

hours of age he tolerated feedings well and after 24 hours he had normal activity. The infant continued to gain weight and was discharged at the age of 20 days.

Case 5

This 15 year-old primipara with eclampsia at 32 weeks gestation had received a total of 41.3 gm $MgSO_4$ over a 30-hour period. The first twin weighed 1,431 gm and had a 1-minute Apgar score of 1. He required endotracheal intubation and assisted ventilation for 1 hour before establishing irregular, spontaneous respiratory movements. An umbilical artery catheter was introduced at 90 minutes of age for infusion of 10% dextrose and water and monitoring of acid-base studies and biochemical estimation. The infant had a metabolic and respiratory acidosis which was treated with $NaHCO_3$. Chest roentgenogram at 3 hours of age was consistent with the diagnosis of hyaline membrane disease and the infant expired at 9 hours of age. The second twin weighed 1,502 gm and had an Apgar score of 5 at 1 minute. He breathed spontaneously after birth but was noted to be flaccid and had a weak cry. At 2 minutes of age he stopped breathing and required endotracheal intubation and assisted ventilation for 15 minutes. An umbilical artery catheter was also inserted for further monitoring and biochemical estimations. A chest roentgenogram at 3 hours of age was also consistent with hyaline membrane disease and he expired at 30 hours of age. At autopsy both infants had histological evidence of hyaline membrane disease. The findings are recorded on Table IV.

Case 6

An 18-year-old primipara was admitted with severe preeclampsia and then convulsed in the labor ward before delivery. The duration of the pregnancy was 40 weeks. The mother received 94 gm $MgSO_4$ over a 44-hour period. The female infant weighed 3,711 gm at birth, had a 1-minute Apgar score of 1, and required endotracheal intubation and resuscitation for 5 minutes. The 5-minute Apgar score was 7. The infant was flaccid with a poor, weak cry and incomplete Moro reflex. Muscle tone was noted to be poor and she had a weak grasp and sucking reflex. Chest roentgenogram at 6 hours of age showed incomplete expansion of the right lung. She was treated by intravenous infusion via scalp vein of 10% dextrose and water and at 24 hours was active and sucking well. Her course is indicated on Table V.

RESULTS

Table V presents the maternal management and neonatal outcome of the six term pregnancies included in this study. Table IV presents the maternal management and neonatal course of the 10 preterm and low birth weight infants in this report.

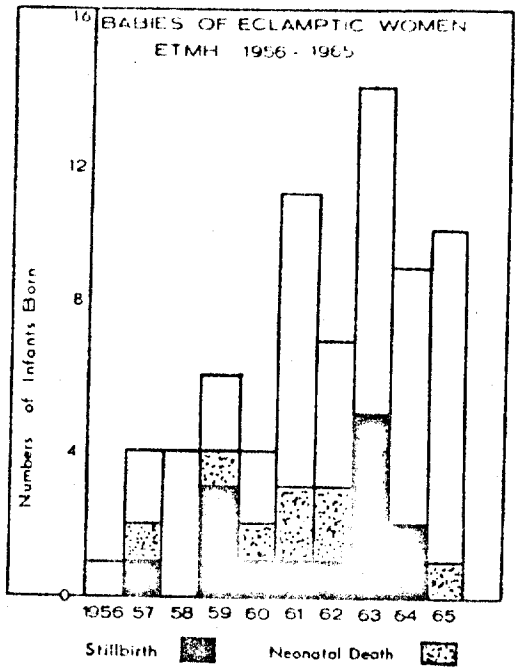


FIG. 1. Number of babies born of eclamptic women at Eugene Talmadge Memorial Hospital between years of 1956 and 1965. (From Holman, G. H., and Lipsitz, P. J.[11] Reproduced by permission of the authors and the editors of Clinical Obstetrics and Gynecology.)

COMMENTS

The maternal and cord magnesium levels are much greater than the normal values obtained in this hospital from uncomplicated pregnancies. It will be noted that from Tables IV and V there is a discrepancy between the maternal serum magnesium level and cord serum levels, but all samples were not obtained simultaneously.

The clinical manifestations seen in these patients are similar to the signs in adults with hypermagnesemia. Randall, *et al.*[5] reviewed the signs and symptoms of hypermagnesemia in adults with chronic renal failure. Elevation of serum level above 4 mEq/l is usually associated with symptoms. Hypotension occurs at levels between 5 and

TABLE IV

MATERNAL MANAGEMENT AND NEONATAL OUTCOME IN PRETERM AND LOW BIRTH WEIGHT INFANTS

Age (Wk)	Gestation (Wk)	MgSO4	Time Infused	Maternal Mg mEq/l	Cord Mg mEq/l	Birth Weight (gm)	Apgar Score	Clinical Course	Serial Mg and Age mEq/l
36	40	33	13	9.1	7.3	2,225	9 at 1 min	Cry and activity normal at birth; normal neonatal period	Not done
30 (Twins)	Unknown	30	12 Stopped 9 hr prior to delivery	3.0	3.1	1,774	8 at 1 min	Both had good cry and normal activity	Not done
						2,011	8 at 1 min		
30	32-34	30	38	Not done	4.7	1,286	1 at 1 min	Hypotonic, cyanotic, endotracheal resuscitation; required O_2; weak cry; normal activity at 48 hr	Not done
24	36	35	15	4.6	6.6	1,835	9 at 1 min	Active, good cry; regular respiration	Not done
22	Unknown	27	8	5.5	6.0	970	2 at 1 min	Flaccid, cyanotic, endotracheal resuscitation; unresponsive, assisted ventilation; died at age 6 hr	Not done
19 (Case 2)	36	21	24	5.3	5.3	2,500	5 at 1 min 10 at 5 min	See detailed report Table III	3.2 at 3 hr 2.5 at 6 hr 2.7 at 24 hr
13 (Case 4)	40	31	20	5.2	4.7	1,980	2 at 1 min	See text	4.0 at 12 hr
15 (Case 5 Twins)	32	41.3	30	5.4	5.6	1,431	1 at 1 min	Endotracheal resuscitation for 1 hr, poor respiratory effort, I.R.D.S.; flaccid, mixed acidosis; expired at 9 hr	5.4 at 2 hr
						1,502	5 at 1 min	Endotracheal intubation for 15 min; I.R.D.S., normal acid-base studies; weak cry, flaccid; Expired at 30 hr	4.4 at 2 hr 4.3 at 15 hr

5 mEq/l. Lessened responsiveness occurs with levels above 5 mEq/l. At levels of 7 to 10 mEq/l depression of deep tendon reflexes is noted with weakness, ataxia, and slurred speech. Respiratory depression is manifest with levels at about 10 mEq/l and coma at levels of 12 to 15 mEq/l. Difficulty in micturation is also noted at levels of 4.5 mEq/l and greater.

The clinical manifestations of the newborn infants delivered from severe preeclamptic or eclamptic mothers treated with $MgSO_4$ are similar to the findings of excess magnesium in adults. Brash[6] in part of a thesis on the effects of toxemia of pregnancy upon the fetus and newborn child emphasized the scanty literature with regard to the prospect of survival of liveborn infants of toxemic mothers. Numerous authors have emphasized the increased likelihood of fetal death, premature birth, and perinatal mortality. She compared 125 full-term, liveborn infants of toxemic mothers with the same number of controls of nontoxemic mothers. The only marked difference in the two groups was a higher incidence of neonatal edema in the full-term infants of toxemic mothers. Abnormal lethargy in some infants of toxemic mothers was more closely correlated with the heavy

sedation of the mother prior to delivery. None of the infants in the toxemic group had the clinical manifestations of flaccidity, weak cry, and poor respiratory effort noted in our group. Alexander[10] in his report gave the Apgar scores of his group of 25 newborns delivered from 24 cases of antepartum eclampsia who were rapidly stabilized with hypotensive drugs, followed by early delivery. Although several newborns had low Apgar scores at 1 minute, he does not describe the clinical manifestations noted in our group of newborns. A recent review article[11] on the effects of toxemia on the fetus and newborn further describes other associated problems in the management of the newborn in toxemic pregnancies.

The manifestations of acute or chronic intra-uterine hypoxia may account for some of the manifestations noted in our group of infants, but these findings would be more in keeping with the effects of excess magnesium on the already compromised newborn of a toxemic mother. In our group it will also be noted that one cannot correlate the Apgar score or the clinical evaluation with the cord magnesium level, nor could the Apgar score be correlated with the clinical manifestations. Usually 24 to 48 hours after birth the infant becomes more responsive and soon assumes normal activity, and there are no further problems in the newborn period. Three liveborn infants of the group reported here expired. One with a birth weight of 970 gm expired at 6 hours of age and at autopsy had unexpanded lungs. The twin newborns, with birth weights of 1,431 gm and 1,502 gm, expired at 9 and 30 hours of age, respectively, and at autopsy there were findings of hyaline membrane disease.

If a toxemic mother is managed with $MgSo_4$ infusion prior to delivery, one would anticipate dealing with a severely

TABLE V

MATERNAL MANAGEMENT AND NEONATAL OUTCOME IN ALL TERM GESTATIONS STUDIED

Maternal Age	*Gestation Weeks*	*$MgSO_4$ gm*	*Time infused*	*Maternal Mg^{++} mEq/l*	*Cord Mg^{++} mEq/l*	*Birth Weight gm*	*Apgar Score*	*Clinical Course*	*Serial Mg^{++} and Age mEq/l*
30	40	16	19	6.6	6.0	2,520	6 at 1 min	Depressed, cyanotic, weak cry; poor response to stimuli; required O_2; recovered full activity at 48 hr	Not done
16	40	50	38 Stopped 15 hr prior to delivery	3.1	4.0	2,840	6 at 1 min.	Delayed onset of respiration; feeble cry; recovered after 24 hr; discharged at 10 days	Not done
17	38	46	19.5	8.0	6.6	2,555	9 at 1 min	Hyperirritable; normal activity	4.3 at 24 hr
17 (Case 1)	40	51.5	33.5	>14.0	11.5	2,820	2 at 1 min	See detailed report Table I	8.1 at 9 hr 6.2 at 15 hr 4.7 at 30 hr 3.9 at 54 hr
18 (Case 3)	41	30	19	6.1	5.3	2,780	5 at 1 min 10 at 5 min	See text	4.2 at 21 hr
18 (Case 6)	40	60.4	44	6.4 Cal. 6.7 mg/100 ml Total prot. 4.6 gm/100 ml	5.9 Cal. 8.7 mg/100 ml Total prot. 5.3 gm/100 ml	3,711	1 at 1 min 7 at 5 min	Required resuscitation by endotracheal intubation for 5 min; flaccid weak cry; poor reflexes; recovered at 24 hr; discharged at 9 days	5.1 at 7 hr 4.0 at 15 hr 4.2 at 32 hr 3.6 at 80 hr

depressed infant at birth. The newborn may then require endotracheal resuscitation and often assisted ventilation for several hours. The cry would be noted to be absent or weak and the newborn would not respond to stimuli as normal infants do. The reflexes should be absent or feeble and the infant would require careful monitoring and intravenous fluids until the sucking reflex becomes stronger and the infant can receive oral feedings.

The serum calcium was not determined routinely in these infants. Further studies will include determination of serum calcium and total proteins. Only about one third of the extracellular magnesium is bound to protein. The amount of extracellular magnesium is small in comparison with the total body content. The central nervous system depression, and peripheral transmission defects of excess magnesium due to the decreased sensitivity of the end plate to the depolarizing action of applied acetylcholine can be completely antagonized by calcium.[12] Animals anesthetized by magnesium will return to consciousness and behave normally a few seconds after intravenous administration of sufficient calcium. Further studies on the effect of intravenous calcium in compromised newborns due to excess magnesium are planned in this institution. In Case 1 the serum calcium was low; but, because of the associated metabolic acidosis, tetany was not observed. The improvement noted could as likely have been due to correction of the acidosis with bicarbonate as to infusion of calcium.

In adults Nelson[13] has found that 70 to 80% of the infused dose of magnesium can be found in the urine within 24 hours after a single intravenous infusion. Whether the neonate excretes the magnesium in the urine in a similar manner has not been studied. Although Randall[7] has noted difficulty in micturition with serum levels of 4.5 mEq/l and greater, it was noted from the newborn records that many of these neonates voided 4 to 6 hours after birth. The renal excretion of magnesium in the newborn will also be further evaluated.

SUMMARY

This report describes 16 cases of hypermagnesemia in neonates of toxemic mothers treated with $MgSO_4$ only. The maternal and fetal serum levels of magnesium were greater than the normal values given in the literature and in our studies. The clinical manifestations in the newborn are similar to adults with hypermagnesemia. Awareness of this problem will help in the management of these infants.

REFERENCES

1. Fishman, R. A.: Neurological aspects of magnesium metabolism. Arch. Neurol., 12:562 1965.
2. Hutchinson, H. T., Nichols, M. M., Kuhn, C. R., and Varicka, A.: Effects of magnesium sulfate on uterine contractility, intrauterine fetus and infant. Amer. J. Obstet. Gynec., 88:747, 1964.
3. Zuspan, F. P.: Treatment of severe pre-eclampsia and eclampsia. Clin. Obstet. Gynec., 9:954, 1966.
4. Bohuon, C.: Microdosage du Magnesium Dans Divers Milieux Biologiques. Clin. Chim. Acta, 7:811, 1962.
5. Bogert, L. J., and Plass, E. D.: Placental transmission. I. The calcium and magnesium content of fetal and maternal blood serum: J. Biol. Chem., 56:297, 1923.
6. Bajpai, P. C., Sugden, D., Ramos, A., and Stern, L.: Serum magnesium levels in the newborn and older child. Arch. Dis. Child., 41:424, 1966.
7. Anast, C. S.: Serum magnesium levels in the newborn. PEDIATRICS, 33:969, 1964.
8. Randall, R. E., Jr., Cohen, M. D., Spray, C. C., Jr., and Rossmeisl, E. C.: Hypermagnesemia in renal failure—etiology and toxic manifestations. Ann. Intern. Med., 61: 73, 1964.
9. Brash, A. A.: The effect of toxaemia of pregnancy upon the foetus and newborn child. Arch. Dis. Child., 24:107, 1949.
10. Alexander, J. A.: A follow-up on infants born of eclamptic mothers. Amer. J. Obstet. Gynec., 95:19, 1966.
11. Holman, G. H., and Lipsitz, P. J.: Effects of toxemic pregnancy on the fetus and neonate. Clin. Obstet. Gynec., 9:922, 1966.
12. Goodman, L. S., and Gilman, A.: The Pharmacological Basis of Therapeutics. New York: The Macmillan Company, p. 803, 1966.
13. Nelson, G.: Personal communication.

J. chron. Dis. 1963, Vol. 16, pp. 201–215. Pergamon Press Ltd. Printed in Great Britain

AN OUTLINE OF MAGNESIUM METABOLISM IN HEALTH AND DISEASE—A REVIEW

IAIN MACINTYRE
Postgraduate Medical School, London

(*Received* 30 *October* 1962)

BASIC CONSIDERATIONS

Chemistry

Magnesium is plentiful and makes up 2.1 per cent of the earth's crust [1]. It occurs in the insoluble form in large masses as magnesite, $MgCO_3$, and as dolomite, $MgCO_3.CaCO_3$. It is also present in less plentiful minerals such as asbestos (a calcium magnesium silicate), and in meerschaum, serpentine and talc, which are also silicates. In the soluble form it is present as carnallite, kainite, schönite and kieserite. Sea water and some spring waters contain magnesium in solution.

Magnesium is usually classified with the alkaline earth metals (Group II A of the Mendeleef classification), although in many ways it has closer resemblance to zinc and cadmium (Group II B). It is a light, white metal of atomic number 12 and atomic weight 24.32. It is extremely reactive and will burn in air, carbon dioxide or the halogens. Like the other metals of the alkaline earths, magnesium readily forms divalent ions, losing its two valency electrons, leaving the ion with the electronic shell of the inert gas (neon, in the case of magnesium) two units lower in atomic number. Ions of higher valency are therefore not known.

The general chemical reactions of magnesium are too well known to need description.

Comparative physiology

Magnesium is invariably present in all living tissues and is essential for life [1]. It is present in chlorophyll in all green plants and it also occurs as a universal micro-constitutent of lower plants. Magnesium is important in some skeletons, for example in echinoderms and crustaceans. In the former the carbonate may make up more than 10 per cent of the skeleton. All tissues of the higher animals contain magnesium, usually in considerable quantities; sodium, potassium and calcium are also major constituents. The occurrence of magnesium can only be understood when these four major electrolytes are considered together.

Implicit in our modern view of the marine origin of life more than a thousand million years ago is the belief that the composition of the sea must have influenced profoundly the composition of vertebrate extracellular fluids. This was first clearly expressed by MACALLUM [2] who compared sea water analyses with the values for

sodium, potassium, calcium and magnesium in the serum from marine invertebrates, elasmobranchs, teleosts and mammals. By expressing his results in terms of 100 parts of sodium, he removed many of the differences in extracellular fluid concentration, which would seem to confound the thesis that extracellular fluid is but modified sea water more or less dilute. The resemblances between sea water and serum are very striking, and whatever exact interpretation is put upon the figures, there can scarcely be any doubt that they do reflect a marine ancestry.

It seems incorrect, however, to confine one's attention to the extracellular fluid. I have collected values [2–4] for the cation content of tissue from various species (Table 1). The absolute concentrations of cations in serum are very variable: in

TABLE 1. THE CATION CONTENT OF TISSUE AND SERUM
The values are expressed as m-equiv/kg (whole tissue) or m-equiv/litre (serum)

	Animal	Habitat	Tissue Na	Tissue K	Tissue Ca	Tissue Mg	Serum Na	Serum K	Serum Ca	Serum Mg
VERTEBRATA	Man	T	30	102	3	19	140	5	5	1.7
	Pig	T	19	102	2	22				
	Dog	T	41	83	3	19	141	5	5	1.9
	Frog	A	24	84	8	19				
	Pike (teleost)	FW	13	106	20	26				
	Cod (teleost)	M					181	10	8	5
	Dogfish (elasmobranch)	M					257	8	8	12
ARTHROPODA	Crab (*Limulus*)	M					386	13	18	83
	Lobster (*Homarus*)	M					393	9	22	13
	Crab (*Astacus*)	FW					152	3	24	5
	Cockroach (*Periplaneta*)	T	46	112			109	17		
MOLLUSCA	Squid (*Loligo*)	M	54	114			354	17		
	Octopod (*Eledone*)	M	81	101	7	25	425	12	23	114
	Mussel (*Anodonta*)	FW	5	11	11	5	15	0.4	11	0.7
ANNELIDA	Worm (*Phascolosoma*)	M	122	106	9	—	378	38	21	—
ECHINODERMATA	Sea cucumber (*Caudina*)	M	191	139	180	78	460	12	21	102
	Sea urchin (*Echinus*)	M					444	10	19	100
COELENTERATA	Jellyfish (*Aurelia*)						429	12	18	100
	Modern ocean						478	10	22	110
	Mid pre-Cambrian		298	104	4	22				
	Freshwater (Hard)						1	0.4	3	1.2
	Freshwater (Soft)						0.7	—	0.5	0.04

A, amphibian; FW, freshwater; M, marine; T, terrestrial.

molluscs, echinoderms and jellyfish, the extracellular fluid closely approaches the composition of the external environment. The other samples of sera depart to a greater or lesser extent from the composition of the ocean, either as it is in modern times or as it was in prehistoric days. On inspection of the tissue content of cations, however, one interesting feature emerges. This is that the concentrations of potassium and magnesium are very similar in most of the species examined, and further, are fairly close to the calculated concentrations of potassium and magnesium in the mid

pre-Cambrian ocean [5]. A very speculative explanation of these resemblances is as follows. When unicellular organisms emerged in the pre-Cambrian period they incorporated in their protoplasm proportions of potassium and magnesium roughly similar to those in the oceans at that time. After immense periods of time multicellular organisms developed, and colonized fresh water and eventually the land. Extreme changes in the extracellular fluid did not occur throughout this period and the cells were able to maintain their original magnesium and potassium concentrations. This must have involved not only an active potassium uptake linked with sodium extrusion but also, as I shall discuss below, possibly an active magnesium transport as well.

This resemblance in tissue electrolyte concentrations in quite different species can scarcely be fortuitous. That in these concentrations, animals, including man, bear within their cells a primeval legacy of the pre-Cambrian oceans seems to me a reasonable hypothesis (Fig. 1).

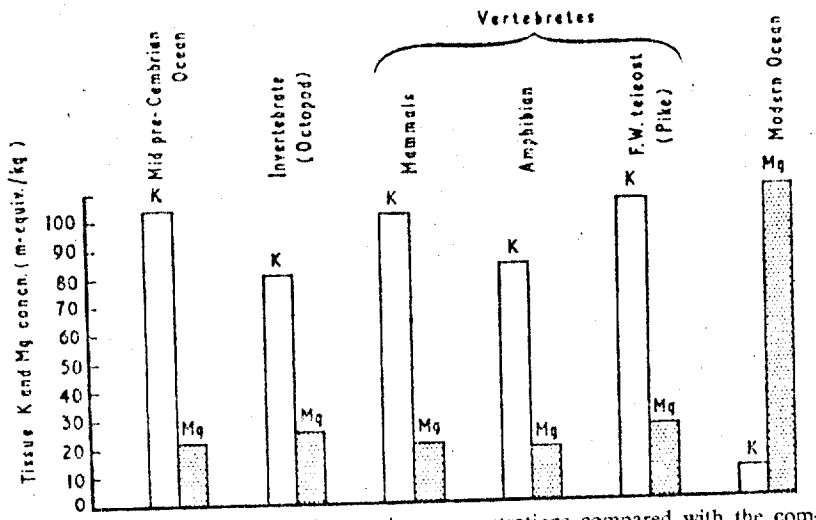


FIG. 1. Tissue magnesium and potassium concentrations compared with the composition of the modern and pre-Cambrian oceans.

Biochemistry

Magnesium is an activator for a very large number of enzymes. This is perhaps to be expected from the preceding account. So many enzymes are likely to be affected that it is quite impossible to predict the *in vivo* effects of magnesium deficiency from the numerous *in vitro* actions of the ion.

WACKER and VALLEE [6] have summarized the enzyme systems for which magnesium is important. They may be classified as follows:

(1) The alkaline phosphatases and the pyrophosphatases are activated by magnesium and inhibited by beryllium. Prostatic acid phosphatases is also activated by magnesium.

Magnesium activates all enzymes transferring phosphate from ATP or to ADP. Since ATP forms a magnesium complex it has been suggested that this may be the

enzyme substrate. So fundamental and widespread are the reactions involving ATP and ADP that magnesium must influence almost all processes of life.

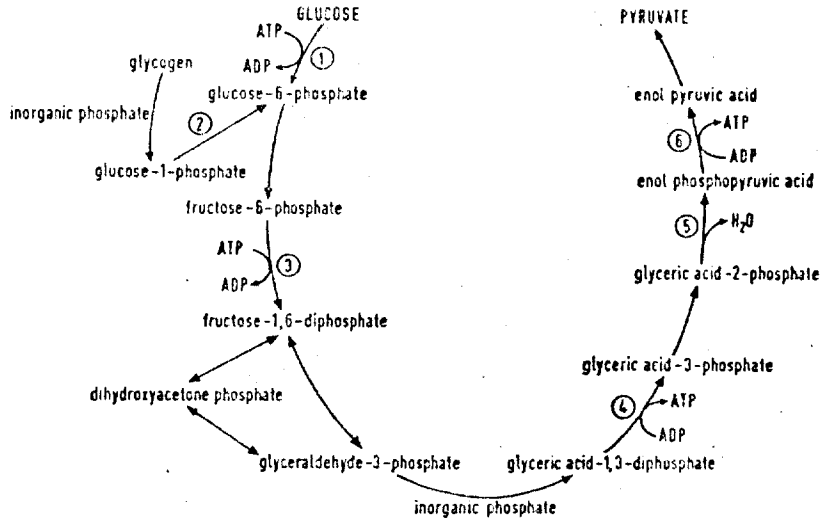
Examples of the phosphate-transferring enzymes activated by magnesium are: hexokinase, fructokinase, creatine transphosphorylase, phosphopyruvic transphosphorylase, phosphoglyceric transphosphorylase, acetyl transphosphorylase, diphosphopyridine nucleotide phosphorylase and phosphoglucomutase.

(2) Magnesium ions are necessary whenever diphosphothiamine is required [7]. Yeast carboxylase, mammalian heart carboxylase and the pyruvic oxidase system of brain are among this group.

(3) Enolase, an enzyme of great importance and of very wide distribution, catalyses the interconversion of 2-phosphoglyceric and phosphoenol-pyruvic acids, an energy-rich phosphate bond being generated in the process. The enzyme requires the presence of magnesium in fairly high concentrations and is believed to occur naturally as the magnesium complex.

(4) Some peptidases, such as leucine aminopeptidase, require magnesium.

(5) Magnesium is necessary for oxidative phosphorylation by isolated mitochondria. VITALE, NAKAMURA and HEGSTED [8] claimed that mitochondria, obtained from rats that had been made magnesium deficient, showed impaired oxidative phosphorylation. However, BEECHEY, ALCOCK, HANNA and MACINTYRE [9] were quite unable to confirm these results and this supposed example of an *in vivo* correspondence with *in vitro* results (after only a few days of deficiency, and with most of the body magnesium still intact) must be regarded with some scepticism.



Reaction	Enzyme	Reference
1.	Hexokinase	BAILEY *et al.* [74]
2.	Phosphoglucomutase	LELOIR *et al.* [75]
3.	6-Phosphofructose kinase	AXELROD *et al.* [76]
4.	Phosphoglycerate kinase	AXELROD *et al.* [77]
5.	Enolase	WARBURG *et al.* [78]
6.	Pyruvate phosphokinase	KORNBERG *et al.* [79]

FIG. 2. The stages of glycolysis activated by magnesium ions.

OPARIN [10] explains the almost universal occurrence of the reactions of glycolysis in living cells as a survival of a primary set of anaerobic energy-yielding reactions from the time when the earth's atmosphere contained little oxygen. If the views on the evolutionary origin of intracellular magnesium already expressed are correct, one would expect that many of the reactions of glycolysis would be activated by magnesium ions. That this is so is shown by Fig. 2. This is only one of the many magnesium-activated reaction sequences which could be illustrated.

Pharmacology

Nervous system. Magnesium has a peripheral, curare-like action, antagonised by cholinesterase inhibitors and in addition, a central depressing effect in higher dosages which is antagonised by analeptics. Magnesium has been used as a general anaesthetic for major operations [11].

The peripheral effects of magnesium have been attributed to a decreased liberation of acetylcholine at the neuromuscular junction and sympathetic ganglia. This can be antagonised by calcium.

Cardiovascular system. With sufficient dosage, magnesium produces a fall in blood pressure in experimental animals. It stops the heart in diastole at plasma concentrations of 27–44 m-equiv/litre. Lower plasma levels in dogs increase the PR interval, the duration of the QRS complex and the height of the T waves.

Bradycardia may be produced by administration of magnesium and experimental ventricular arrhythmias produced by digitalis can be reversed.

PHYSIOLOGY

Magnesium is the fourth most abundant cation in the body and an adult 70 kg man has a total body content of about 2000 m-equiv. of magnesium. This is to be com-

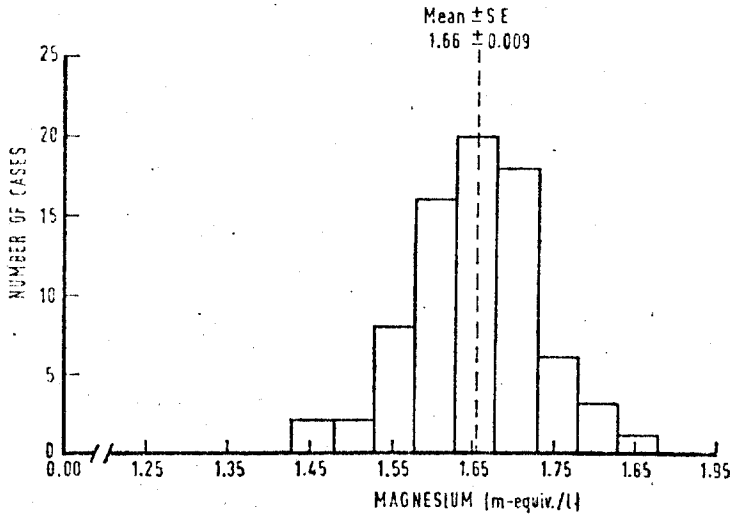


FIG. 3. Histogram of the plasma magnesium concentration in 76 healthy adults.

pared with 60,000 m-equiv. of calcium, 3000 m-equiv. of potassium and rather more than 5,500 m-equiv. of sodium. As is the case with sodium, about half of the total magnesium is in the skeleton; the remaining magnesium is almost all intracellular. However, although the total magnesium in extracellular fluid represents only about 1 per cent of the magnesium in the body, the plasma magnesium is maintained constant within narrow limits [12]; and Fig. 3. One can find reports which seem to indicate that the plasma magnesium varies quite widely but this is evidence of faulty analysis rather than homeostatic failure.

Adults ingest roughly 20–25 m-equiv. of magnesium a day. About one-third of this is absorbed and excreted in the urine [13, 14].

Specific factors such as protein intake [15], the administration of growth hormone [16], large doses of vitamin D [17] and of antibiotics [18], have been shown to influence magnesium absorption from the gut. But no single factor has been shown to play a role in the absorption of magnesium similar to that of vitamin D in calcium absorption [19]. The constancy of the plasma magnesium is evidence of a homeostatic control, but we have no idea of its nature. Such a mechanism might involve the kidney and the gut, and it may be useful to summarize some aspects of the urinary and faecal excretion of magnesium.

The renal excretion of magnesium

CHESLEY and TEPPER [20] showed that magnesium clearance, corrected for protein binding, increased as a linear function of serum magnesium and approached the inulin clearance at high plasma levels of magnesium. It was suggested that there was normally almost maximal tubular resorption of magnesium. These workers also confirmed earlier findings [21–24] that intravenous infusion of magnesium in man increased calcium excretion in the urine.

BERGLUND and FORSTER [25] studied the aglomerular kidney of the marine teleost, *Lophius americanus*. They suggested that a separate transport system for divalent cations must exist to explain their findings. Under their conditions the divalent cation with the higher excretion rate competitively depressed transfer of the other ion. ALCOCK and MACINTYRE [26, 27] suggested that a common tubular resorptive system might also exist in the mammalian kidney. They based this suggestion on their finding of marked hypocalciuria in magnesium-deficient rats (Fig. 4) despite the presence of hypercalcaemia. A similar suggestion was also made on the basis of infusion and stop-flow studies in the dog [28]. The SAMIY group [28] also noted that if radioactive calcium and magnesium were simultaneously injected with inulin, 1–2 min before releasing the ureteral occlusion, they appeared together in the urine prior to the appearance of inulin. Although such observations indicate movement of these ions in both directions across the tubular epithelium, they do not imply active or net secretion as claimed by GINN *et al.* [29].

No experimental evidence of magnesium secretion by the mammalian tubule has so far been produced. Our present concept is that magnesium is excreted by the kidney by glomerular filtration coupled with almost complete tubular resorption. Calcium may compete for this resorptive path but we do not know any details of this process nor can we localize it accurately in the nephron.

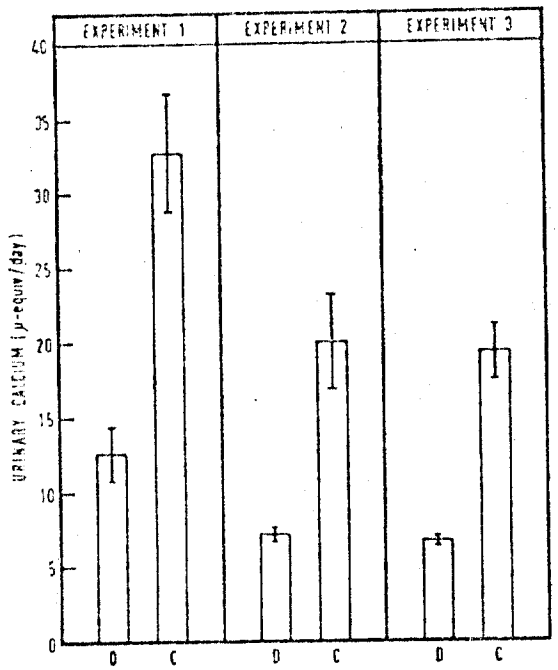


FIG. 4. The urine calcium excretion in magnesium-deficient rats. The height of the columns represents the mean excretion; S.E.M. is also indicated. D, magnesium-deficient rats; C, control rats.

Intestinal absorption of magnesium

Our knowledge of the factors controlling the intestinal absorption of magnesium is even more limited. The site of absorption of magnesium in man [30], and animals [31] appears to be the proximal part of the small intestine. Some of the factors which influence magnesium absorption have been noted above. A common transport mechanism for the transfer of calcium and magnesium across the intestinal wall, similar to the suggested system in the renal tubule, has been postulated by ALCOCK and MACINTYRE [26, 27] on the basis of balance experiments in magnesium-deficient and calcium-deficient rats. This is consistent with the *in vitro* studies of SCHACHTER and ROSEN [32]. They showed that calcium transport across the intestinal wall was depressed by the presence of magnesium ions in the medium. A common intestinal transport system has clinical implications in man and may explain hypomagnesaemia in calves. This aspect will be discussed below.

Despite some recent progress we remain ignorant of the main factors responsbile for the control of magnesium in the body; the discovery of the mechanisms of magnesium homeostasis is the most pressing problem in this field.

Intracellular magnesium

Table 2 shows the electrolyte concentrations in various rat, amphibian and human tissues. Magnesium is known to form strong complexes with many cellular con-

TABLE 2. TISSUE ELECTROLYTE CONCENTRATIONS

Tissue		Total (m-equiv/kg dry fat-free solids)	Intracellular (m-equiv/litre intracellular water)
Rat skeletal muscle	Mg	100	33
	K	450	150
	Ca	14	4
	Na	92	6
	Cl	60	—
Frog skeletal muscle	Mg	—	23 (7)
	K	—	140 (122)
	Ca	—	3 (2)
Human skeletal muscle	Mg	75	28
	K	430	150
	Ca	10	3
	Na	150	18
	Cl	85	—
Rat liver	Mg	82	32
	K	352	140
	Ca	5	1

Values for rat skeletal muscle and rat liver are calculated from MACINTYRE and DAVIDSSON [47] assuming 78 per cent water in muscle and 2.5 litres intracellular fluid/kg of liver. Values for human muscle are calculated from MACINTYRE, HANNA, BOOTH and READ [53] and from TALSO *et al.* [72]. The frog tissue values are from NANNINGA [73]. Values in brackets are the calculated free electrolyte concentrations.

stituents, especially ATP. When these complexes are taken into account it appears that the concentration of free magnesium ions in frog muscle is about one-third of the total (Table 2). It seems likely that this is also true of other tissues and this may have led to the belief that magnesium exists in the cells in some stable non-exchangeable form. This does not follow from the fact that magnesium is tightly bound by many compounds in the cell. The idea is shown to be untenable by the following experiment. Carrier-free ^{28}Mg was injected into six rats. The rats were killed at intervals over several days; the organs were removed and assayed for radioactive and stable magnesium. When the specific activities are plotted against time (Fig. 5) it can be seen that radioactive magnesium penetrates most tissues in the body, in some cases very rapidly. Thus, the curve for the specific activity of liver magnesium is very similar to the curve obtained for liver potassium when radioactive potassium is injected [33]. As also occurs with potassium, the brain magnesium exchanges at a slower rate; in contradistinction to potassium, however, the magnesium in skeletal muscle exchanges even more slowly than the magnesium in brain. The situation is quite different in cardiac muscle [34] where the uptake of injected radioactive magnesium is greater than in any other tissue.

It is of interest to consider whether the distribution of magnesium between intra- and extra-cellular fluids is explicable on the basis of passive diffusion through water-filled membrane pores. If this is so, then the electrochemical potential of magnesium will be equal inside and outside of the cell. Since the membrane potential can be approximated in many tissues from the potassium concentration gradient we can

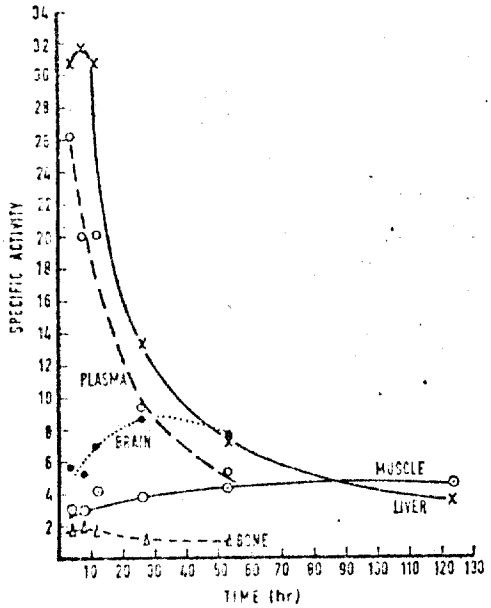


FIG. 5. Tissue specific activities in the rat after injection of ^{28}Mg.

calculate that the membrane potential, E, of a liver cell *in vivo* will equal:

$$60 \log \frac{[\text{Intracellular potassium activity}]}{[\text{Extracellular potassium activity}]}$$ This gives a value of about 90mv.

The electrochemical potential of magnesium in the intracellular fluid, $\bar{\mu}_{in}$, is: $RT \ln a_{in} + zF\psi + I$ where R, T, z and F have the conventional meaning, a_{in} is chemical activity, ψ electrical potential and I a constant [35]. If magnesium distribution is passive, $\bar{\mu}_{in} - \bar{\mu}_{out} = 0$, whence

$$\log x = \frac{zF\psi}{2.3RT} = 16.7\, z\psi,$$

where x is the equilibrium distribution ratio and $\bar{\mu}_{out}$ the electrochemical potential of extracellular magnesium. This solves to $x = 1000$. But $x = 25$ in rat liver, even assuming all intracellular magnesium to be free. There is a 40-fold discrepancy, therefore, and we must conclude that magnesium is actively transported out of liver cells (and presumably other body cells) or that it crosses the membrane by exchange diffusion [35] or that some of the basic simplifying assumptions inherent in the above discussion are wrong.

These possibilities can be sorted out by experiments with tissue slices. It is to be hoped this will be accomplished soon—a sensitive analytical method and a suitable isotope are both available.

MAGNESIUM DEFICIENCY

Naturally-occurring deficiency in animals

Cows, sheep and horses are prone to a disease characterized by hyperexcitability and

associated with a reduction in serum magnesium. Two forms of the disease are known in cattle. The first occurs in calves who have been kept on a milk diet exclusively, for a prolonged period; the second form may affect older animals turned out to spring pasture (grass staggers). The annual incidence in suckling calves has been placed as high as 5 per cent [36].

SJOLLEMA and SEEKLES [37] were the first to recognize hypomagnesaemia in cattle. The disease was referred to as 'kopziekte'. This term was translated a year later as 'grastetanie' [38]. Had 'kopziekte' been translated as 'grass epilepsy' much subsequent confusion in clinical medicine might have been avoided. The term 'tetany' implies incorrectly that the syndromes of hypomagnesaemia and hypocalcaemia are identical.

The cause of the deficiency in the adult cow is unknown but dietary phytate may play a role by preventing the intestinal absorption of magnesium [39]. ALCOCK and MACINTYRE [27] suggest that deficiency in milk-fed calves is produced by the very high calcium intake. The excess calcium in milk presumably competes with magnesium for absorption by a common resorptive path. The results of an experiment carried out by Dr. R. ALLCROFT of the Central Veterinary Laboratory, Weybridge, are shown in Fig. 6. She found when she supplemented the exclusively milk diet of

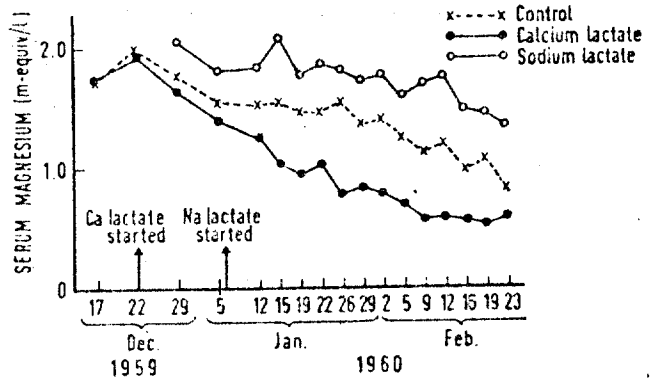


FIG. 6. Hypomagnesaemia in milk-fed calves. All calves fed on whole milk at 10 per cent of body weight per day.
×- - - -× controls (four animals), fed milk only.
●——● extra calcium group (three animals), fed 6 oz of calcium lactate daily mixed in milk.
o——o sodium lactate group (three animals), fed 4 oz of sodium lactate daily mixed in milk.

calves with further calcium that hypomagnesaemia occurred earlier and became more severe.

Experimental deficiency

Experimental deficiencies have been produced in rats [40], mice [41], rabbits [42], dogs [43] and guinea-pigs [44]. The rat has been studied most and the effects of deficiency are discussed below [40, 45–47].

Clinical changes. After about a week or ten days on a magnesium-deficient diet,

rats develop an intense peripheral vasodilation. This slowly fades and the animals become liable to epileptiform seizures which may be fatal and which are easily brought on by high pitched noises. The animals then pass into a cachectic phase and die after a few weeks. The syndrome is quite distinct from hypocalcaemia. This was emphasized by GREENBERG and TUFTS [48] although, following SIOLLEMA and SEEKLES, they continued to use the term 'tetany.' GREENBERG and TUFTS were able to distinguish hypomagnesaemia and hypocalcaemia in the rat both clinically and pharmacologically; tubocurarine inhibited the symptoms of hypocalcaemia but exacerbated those of magnesium deficiency.

Biochemistry. Despite earlier claims to the contrary, modern analytical methods show that there is a slow decline in muscle magnesium. In the 100 g rat the rate of decline is about 2 per cent per week. Surprisingly there is also a decline in muscle potassium, despite the maintenance of normal serum potassium levels and despite adequate potassium in the diet [47, 49, 50]. The cause of the secondary potassium deficiency is so far unknown.

Most workers have found a decline in the bone magnesium concentration [45, 47, 51]. However, although the 35 g weanling rat may lose between one-sixth and one-third of its total skeletal magnesium, in those weighing 100 g and in older rats the main cause of the decline in skeletal magnesium concentration is deposition of bone which is very poor in magnesium. BLAXTER [52] showed that adult magnesium-deficient cattle lost none of their skeletal magnesium, while MACINTYRE, HANNA, BOOTH and READ [53] found minimal reduction in skeletal magnesium concentration in an adult magnesium-deficient subject. This indicates that the concept that skeletal magnesium can always act as a readily available reservoir is untenable. The studies with radioactive magnesium support this conclusion.

As would be expected, there is a fall in plasma magnesium concentration. This is accompanied by hypercalcaemia [47, 50]. The reason for the latter phenomenon is unknown.

Morbid anatomy. Various histological changes have been described but nephrocalcinosis is the most uniform feature. HESS, MACINTYRE, ALCOCK and PEARSE [54] described the production, after only a week of magnesium deficiency, of a renal tubular lesion which starts with mitochondrial swelling.

Magnesium deficiency in man

Hypomagnesaemia is a common occurrence in clinical medicine. That it is often unrecognized is due to the fact that magnesium is still rarely estimated. As with potassium before the advent of the flame photometer, this omission may be without much consequence; sometimes, however, undetected hypomagnesaemia significantly contributes to the underlying illness and occasionally may even prove fatal.

HANNA, HARRISON, MACINTYRE and FRASER [55] make the following points about the clinical picture:

(1) Tetany, in the sense of painful peripheral muscle cramps, does not occur in pure magnesium deficiency. This was also the view of FLINK, STUTZMAN, ANDERSON, KONIG and FRASER [56], but has been contested by WACKER, VALLEE and ULMER [57]. This confusion may have arisen from the continued use of the term tetany, by KRUSE *et al.* [40], to describe magnesium deficiency in the rat, after the term 'gras-

tetanie' was applied to the disease in cattle by SEEKLES *et al.* [38]. However, as has been mentioned, the syndrome in the rat was recognized as different from that produced by hypocalcaemia [48]. This fact was not usually appreciated by clinical researchers who looked for hypomagnesaemia in patients who exhibited tetany in the usual clinical sense of carpo-pedal spasm. Since most of these patients were suffering from multiple electrolyte and nutritional disturbances [58], it is hardly surprising that a low plasma magnesium was sometimes found. In the reviewer's opinion, most modern workers would now agree that the syndromes of hypomagnesaemia and hypocalcaemia are as different in man as in the experimental animal. If one is to use the term tetany for the magnesium state, and I believe this is best avoided, it is tetany in which (i) cramps do not occur; (ii) Trousseau's sign is negative; and (iii) apart from a positive Chvostek's sign, convulsive seizures may be the only manifestation.

(2) There is a group of non-specific signs and symptoms which are attributable to magnesium deficiency when they are promptly removed by therapy. Thus, muscular weakness, vertigo, mental changes (especially extreme irritability and aggressiveness), gross tremors and non-specific EEG and ECG changes may occur in various combinations. Sometimes the picture is sufficiently characteristic to allow of a clinical diagnosis. More usually, the picture is as non-specific as that of most cases of potassium deficiency.

(3) The characteristic and most serious feature of severe magnesium deficiency is a liability to severe and potentially fatal epileptiform seizures.

Many of these features were first recognized by FLINK *et al.* [56] despite their lack of the modern flame spectrophotometric methods which now make magnesium estimation so simple.

Some of the clinical conditions in which magnesium deficiency may occur are summarized below:

(1) The malabsorption syndrome. BOOTH, HANNA and MACINTYRE [59], found that of 35 cases of steatorrhoea 10 were hypomagnesaemic, 5 to a marked degree (Fig. 7). As expected, this is due to loss of magnesium in the stools [53, 55, 60].

MACINTYRE *et al.* [53] took bone and muscle biopsies from a case of idiopathic steatorrhoea and demonstrated that cellular depletion of magnesium may occur with little or no change in skeletal content.

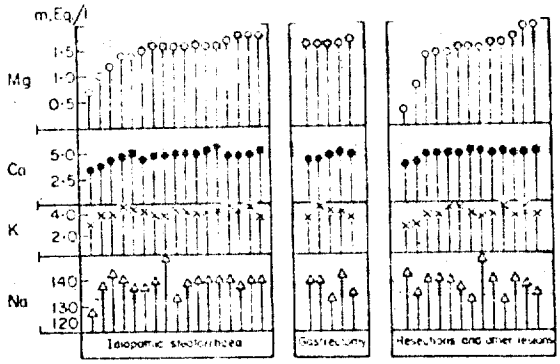


FIG. 7. Plasma electrolytes in the malabsorption syndrome.

(2) Primary hyperparathyroidism: Several authors have studied magnesium metabolism in parathyroid tumour [61–65]. There are two abnormalities of magnesium metabolism:

(a) Hypermagnesuria is usual, but not invariable. This may cause a negative magnesium balance and if this is marked, hypomagnesaemia may occur preoperatively.

(b) Hypomagnesaemia develops after the tumour is removed. This is probably due to deposition of magnesium in bone, [65] and Fig. 8, and is most obvious in cases with bone disease.

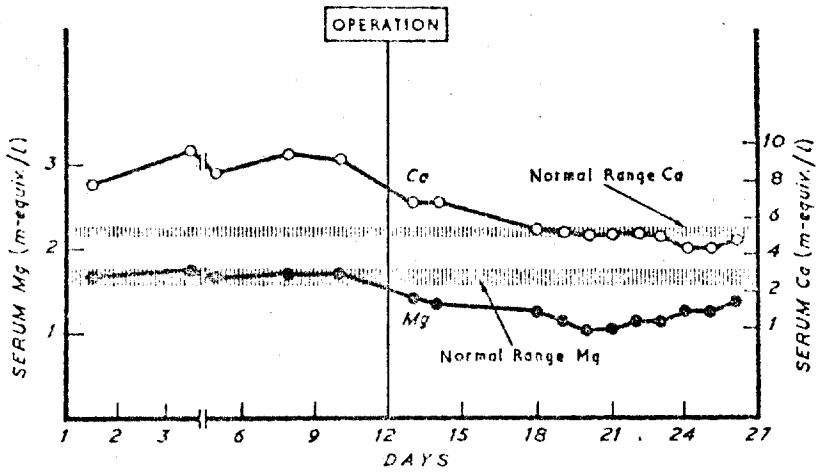


FIG. 8. Plasma calcium and magnesium levels in a case of primary hyperparathyroidism.

Magnesium therapy may come to be regarded as an essential part of the management of the post-operative phase of hyperparathyroidism. It is our practice to give oral post-operative magnesium supplements of 1–1.5 m-equiv./kg/day. If the plasma magnesium is low before operation magnesium therapy is essential to avoid the risk of potentially fatal seizures in the post-operative phase.

(3) Prolonged or severe loss of body fluids [56].

(4) Chronic alcoholism. FLINK *et al.* [56] reported that patients with delirium tremens have low serum magnesium concentrations. This should not be taken to imply that magnesium deficiency is the cause of delirium tremens: this is certainly not so. However, there is no doubt that severe magnesium deficiency does occur in some chronic alcoholics and that, as in other patients, it may produce mental changes and require effective treatment.

(5) Diuretic therapy, especially with mersalyl and chlorthiazide [66].

(6) Hypercalcaemia from any cause. Cases of carcinoma of the breast with hypercalcaemia may have a low plasma magnesium [67].

(7) Renal tubular acidosis [55].

(8) Tubular necrosis in the diuretic phase [68].

(9) Portal cirrhosis [69].

(10) Primary aldosteronism [70].

Treatment of magnesium deficiency in man

MACINTYRE [71] advances the following reasons for treating severe hypomagnesaemia: it may produce fatal epileptiform seizures; it produces rapid renal damage in all species where this has been investigated (this does not yet include man); magnesium activates so many enzyme systems that to restore a normal plasma magnesium concentration is as rational as to restore oxygen saturation in anoxaemia.

If hypomagnesaemia is severe (0.8 m-equiv./litre or less), treatment may start with intravenous supplements. As much as 2 m-equiv./kg can safely be given over 4 hr and can be repeated 24 hr later. Usually, however, repletion can be achieved by oral supplements of about 1 m-equiv./kg/day. Occasionally, larger supplements, up to 4 m-equiv./kg/day, may be required. The chloride is preferred for intravenous injection but, where large amounts have to be given by mouth, a mixture of equivalent amounts of the chloride and citrate may be used.

CONCLUSION

Many of the concepts advanced here may shortly be overtaken by new facts, so rapid is progress in this field. If, however, these hypotheses help to unify our understanding of magnesium metabolism and if they suggest further lines of study, they will have served their purpose.

Acknowledgment—I am grateful to Dr. R. ALLCROFT for allowing me to quote from her unpublished work the results shown in Fig. 6.

REFERENCES

1. FEARON, W. R.: *An Introduction to Biochemistry* (3rd Ed.). Heinemann, London, 1948.
2. MACALLUM, A. B.: *Proc. Roy. Soc. B.* 82, 602, 1910.
3. BISHOP, D. W., BROWN, F. A., JAHN, T. L., PROSSER, C. L. and WULFF, V. J.: In *Comparative Animal Physiology*, Ed. by PROSSER, C. L. W. B. Saunders, Philadelphia, 1950.
4. KATZ, J.: *Pflüg. Arch. ges. Physiol.* 63, 1, 1896.
5. CONWAY, E. J.: *Proc. Roy. Irish Acad. B.* 48, No. 9, 161, 1943.
6. WACKER, W. E. C. and VALLEE, B. L.: *New Engl. J. Med.* 259, 431 and 475, 1958.
7. BALDWIN, E.: *Dynamic Aspects of Biochemistry* (2nd Ed.). Cambridge University Press, 1952.
8. VITALE, J. J., NAKAMURA, M. and HEGSTED, D. M.: *J. biol. Chem.* 228, 573, 1957.
9. BEECHEY, R. B., ALCOCK, N., HANNA, S. and MACINTYRE, I.: *Biochem. J.* 71, 18P, 1959.
10. OPARIN, A. I.: *The Origin of Life on the Earth* (3rd Ed.). Oliver & Boyd, Edinburgh, 1957.
11. PECK, C. H. and MELTZER, S. J.: *J. Amer. med. Ass.* 67, 1131, 1916.
12. ALCOCK, N., MACINTYRE, I. and RADDE, I.: *J. clin. Path.* 13, 506, 1960.
13. TIBBETTS, D. M. and AUB, J. C.: *J. clin. Invest.* 16, 491, 1937.
14. LEICHSENRING, J. M., NORRIS, L. M. and LAMISON, S. A.: *J. Nutr.* 45, 477, 1951.
15. MCCANCE, R. A., WIDDOWSON, E. M. and LEHMANN, H.: *Biochem. J.* 36, 686, 1942.
16. HANNA, S., HARRISON, M. T., MACINTYRE, I. and FRASER, R.: *Brit. med. J.* 2, 12, 1961.
17. HANNA, S.: *Metabolism* 10, 735, 1961.
18. HEGGENESS, F. W.: *J. Nutr.* 68, 573, 1959.
19. NICOLAYSEN, R.: *Biochem. J.* 31, 122, 1937.
20. CHESLEY, L. C. and TEPPER, I.: *J. clin. Invest.* 37, 1362, 1958.
21. MENDEL, L. B. and BENEDICT, S. R.: *Amer. J. Physiol.* 25, 1, 1909.
22. HELLER, B. I., HAMMARSTEN, J. F. and STUTZMAN, F. L.: *J. clin. Invest.* 32, 858, 1953.
23. WOMERSLEY, R. A.: *Clin. Sci.* 15, 465, 1956.
24. HAMMARSTEN, J. F., ALLGOOD, M. and SMITH, W. O.: *J. appl. Physiol.* 10, 476, 1957.
25. BERGLUND, F. and FORSTER, R. P.: *J. gen. Physiol.* 41, 429, 1958.
26. ALCOCK, N. and MACINTYRE, I.: *Biochem. J.* 76, 19P, 1960.
27. ALCOCK, N. and MACINTYRE, I.: *Clin. Sci.* 22, 185, 1962.

28. Samiy, A. H. E., Brown, J. L., Globus, D. L., Kessler, R. H. and Thompson, D. D.: *Amer. J. Physiol.* **198**, 599, 1960.
29. Ginn, H. E., Smith, W. O., Hammarsten, J. F. and Snyder, D.: *Proc. Soc. exp. Biol., N.Y.* **101**, 691, 1959.
30. Graham, L. A., Caesar, J. J. and Burgen, A. S. V.: *Metabolism* **9**, 646, 1960.
31. Aikawa, J. K.: *Proc. Soc. exp. Biol., N.Y.* 100, 293, 1959.
32. Schachter, D. and Rosen, S. M.: *Amer. J. Physiol.* 196, 357, 1959.
33. Noonan, T. R., Fenn, W. O. and Haege, L.: *Amer. J. Physiol.* **132**, 474, 1941.
34. Brandt, J. L., Glaser, W. and Jones, A.: *Metabolism* **7**, 355, 1958.
35. Kolfold-Johnsen, V. and Ussing, H. H.: In *Mineral Metabolism*, 1A, p. 169. Ed. by Comar, C. L. and Bronner, F. Academic Press, New York, 1961.
36. Blaxter, K. L. and Sharman, G. A. M.: *Vet. Rec.* 67, 103, 1955.
37. Sjollema, B. and Seekles, L.: *T. Diergeneesk.* **56**, 979, 1929.
38. Seekles, L., Sjollema, B. and van der Kaay, F. C.: *T. Diergeneesk.* **57**, 1229, 1930.
39. Roberts, A. H. and Yudkin, J.: *Nature, Lond.* **185**, 823, 1960.
40. Kruse, H. D., Orent, E. R. and McCollum, E. V.: *J. biol. Chem.* **96**, 519, 1932.
41. Leroy, J.: *C. R. Soc. Biol., Paris* **94**, 431, 1926.
42. Kunkel, H. O. and Pearson, P. B.: *J. Nutr.* 36, 657, 1948.
43. Orent, E. R., Kruse, H. D. and McCollum, E. V.: *Amer. J. Physiol.* **101**, 454, 1932.
44. O'Dell, B. L., Morris, E. R. and Regan, W. O.: *J. Nutr.* **70**, 103, 1960.
45. Watchorn, E. and McCance, R. A.: *Biochem. J.* **31**, 1379, 1937.
46. Tufts, E. V. and Greenberg, D. M.: *J. biol. Chem.* **122**, 693, 1938.
47. MacIntyre, I. and Davidsson, D.: *Biochem. J.* **70**, 456, 1958.
48. Greenberg, D. M. and Tufts, E. V.: *Amer. J. Physiol.* **121**, 416, 1938.
49. Coulove, E., Holliday, M. A., Schwarz, R. and Wallace, W. M.: *Amer. J. Physiol.* **167**, 665, 1951.
50. Whang, R. and Welt, L. G.: *Clin. Res.* **10**, 68, 1962.
51. Duckworth, J., Godden, W. and Wapnock, G. M.: *Biochem. J.* **34**, 97, 1940.
52. Blaxter, K. L.: *Ciba Symp. Bone Structure and Metabolism*, p. 117. Ed. by Wolstenholme, G. E. W. and O'Connor, C. M. Churchill, London, 1956.
53. MacIntyre, I., Hanna, S., Booth, C. C. and Read: *Clin. Sci.*, **20**, 297, 1961.
54. Hess, R., MacIntyre, I., Alcock, N. and Pearse, A. G. E.: *Brit. J. exp. Path.* **40**, 80, 1959.
55. Hanna, S., MacIntyre, I., Harrison, M. and Fraser, R.: *Lancet* **2**, 172, 1960.
56. Flink, E. B., Stutzman, F. L., Anderson, A. R., Konig, T. and Fraser, R.: *J. Lab. clin. Med.* **43**, 169, 1954.
57. Wacker, W. E. C., Vallee, B. L. and Ulmer, D. D.: *New Engl. J. Med.* **262**, 155, 1960.
58. Randall, R. E., Rossmeisl, E. C. and Bethea, K. H.: *Ann. intern. Med.* **50**, 257, 1959.
59. Booth, C. C., Hanna, S. and MacIntyre, I. (unpublished).
60. Fletcher, R. F., Henly, A. A., Sammons, H. G. and Soliee, J. R.: *Lancet* **1**, 522, 1960.
61. Bulger, H. A. and Gausmann, F.: *J. clin. Invest.* **12**, 1135, 1933.
62. Barnes, B. A., Krane, S. M. and Cope, O.: *J. clin. Endocr.* **17**, 1407, 1957.
63. Potts, J. T. and Roberts, B.: *Amer. J. med. Sci.* **235**, 206, 1958.
64. Agna, J. W. and Goldsmith, R. E.: *New Engl. J. Med.* **258**, 222, 1958.
65. Hanna, S., North, K. A. K., MacIntyre, I. and Fraser, R.: *Brit. med. J.* **2**, 1253, 1961.
66. Smith, W. O., Kyriakopoulos, A., Mock, D. C. and Hammarsten, J. F.: *Clin. Res.* **7**, 162, 1959.
67. MacIntyre, I. and Radde, I. (unpublished).
68. Radde, I., MacIntyre, I. and Shackman, R. (unpublished).
69. Stutzman, F. L. and Amatuzio, D. S.: *J. Lab. clin. Med.* **41**, 215, 1953.
70. Mader, I. J. and Iseri, L. T.: *Amer. J. Med.* **19**, 976, 1955.
71. MacIntyre, I.: *Scientific Basis of Medicine Annual Reviews.* In press.
72. Talso, P. J., Spaltford, N. and Blaw, M.: *J. Lab. clin. Med.* **41**, 281, 1953.
73. Nanninga, L. B.: *Biochim. biophys. Acta.* **54**, 338, 1961.
74. Bailey, K. and Webb, E. C.: *Biochem. J.* **42**, 60, 1948.
75. Leloir, L. F., Trucco, R. E., Cardini, C. E., Paladini, A. and Caputto, R.: *Arch. Biochem* **19**, 339, 1948.
76. Axelrod, B., Saltman, P., Bandurski, R. S. and Baker, R. S.: *J. biol. Chem.* **197**, 89, 1952.
77. Axelrod, B. and Bandurski, R. S.: *J. biol. Chem.* **204**, 939, 1953.
78. Warburg, O. and Christian, W.: *Biochem. Z.* **310**, 384, 1941.
79. Kornberg, A. and Pricer, W. E.: *J. biol. Chem.* **193**, 481, 1951.

American Journal of Physiology
Vol. 216, No. 6, June 1969. Printed in U.S.A.

Renal handling of magnesium in the dog

SHAUL G. MASSRY, JACK W. COBURN, AND CHARLES R. KLEEMAN (With the Technical Assistance of Lloyd W. Chapman and Miriam Bick)
Cedars-Sinai Medical Research Institute and Departments of Medicine, Cedars-Sinai Medical Center, Los Angeles 90048, Veterans Administration Center, and UCLA School of Medicine, Los Angeles, California 90024

Massry, Shaul G., Jack W. Coburn, and Charles R. Kleeman. *Renal handling of magnesium in the dog.* Am. J. Physiol. 216(6): 1460–1467. 1969.—Renal handling of Mg was evaluated in 30 dogs receiving 1.0–3.0 μg Mg/min per kg body wt with gradual elevation of diffusible serum magnesium (dSMg) to 12 mg/100 ml. The fraction of filtered Mg excreted (C_{Mg}/C_{Cr}) rose steeply as dSMg increased to 7.0 mg/100 ml; with further rise in dSMg the C_{Mg}/C_{Cr} increased more slowly. Relationship between dSMg and C_{Mg}/C_{Cr} was not different during a rising or falling dSMg or with infusion of $MgCl_2$ or $MgSO_4$. Magnesium reabsorption increased to reach a Tm of 140 μg/min per kg body wt when filtered load was 280 μg/min per kg body wt; during saline diuresis, calcium infusion, or following chronic DCA treatment the Tm for magnesium was 80, 85, and 75 μg/min per kg body wt. When factors known to decrease magnesium reabsorption were superimposed on $MgCl_2$ infusion, C_{Mg}/C_{Cr} was higher for any level of dSMg compared to $MgCl_2$ infusion alone but never exceeded unity; with acute reduction in GFR, C_{Mg}/C_{Cr} invariably fell and magnesium reabsorption remained unchanged. Parathyroid extract administration during $MgCl_2$ infusion caused a fall in C_{Mg}/C_{Cr}. Results indicate that *1)* Mg excretion is determined by filtration and reabsorption without evidence of tubular secretion; *2)* there is a maximum tubular reabsorptive capacity for magnesium; *3)* extracellular volume expansion produced by NaCl infusion, $CaCl_2$ infusion, or chronic DCA treatment are associated with a decrease in magnesium Tm; *4)* parathyroid hormone may directly enhance magnesium reabsorption.

magnesium excretion; magnesium reabsorption; parathyroid hormone; calcium excretion; sodium excretion; magnesium Tm

---

The kidneys play an important role in magnesium homeostasis. Oral or intravenous loads are rapidly excreted (4, 9, 14, 15), and in the magnesium-deficient state or with rigid dietary restriction (6, 11) this ion almost disappears from the urine. Therefore, the delineation of the mechanisms underlying the renal handling of magnesium is important for the understanding of magnesium metabolism.

Certain aspects of the renal transport of magnesium are unclear. It has been suggested that magnesium reabsorption in human subjects (5, 9) and in the dog (4) is at or near a maximal or Tm rate and that any increase in filtered magnesium is quantitatively excreted. However, other studies failed to clarify whether such a reabsorptive Tm exists in the dog (16, 22).

Another perplexing problem is whether magnesium is secreted by the mammalian nephron. Although Averill and Heaton (3) demonstrated magnesium secretion in the rat during magnesium loading, Alfredson and Walser (1), utilizing stop-flow techniques, did not find magnesium secretion in this animal. There is no convincing evidence to indicate that such secretion occurs in dogs or man.

The influence of parathyroid hormone on magnesium excretion is not defined (24). Heaton (13) reported that parathyroid hormone administration increases magnesium excretion in rats. These animals were hypercalcemic, and hypercalcemia is usually associated with augmented urinary calcium and magnesium independent of the effect of parathyroid hormone (10). MacIntyre, Boss, and Troughton (18) found a decrease in both magnesium and calcium excretion in rats following parathyroid hormone administration. It is possible that the change in urinary magnesium is secondary to that of calcium rather than to a direct effect of the hormone on renal magnesium transport.

The present investigation was undertaken to evaluate the characteristics of the renal handling of magnesium in the dog under a variety of test procedures. The results indicate that *1)* magnesium excretion, like sodium and calcium, is determined by filtration and reabsorption alone without evidence for tubular secretion; *2)* there is a maximal tubular reabsorptive capacity (Tm) for magnesium of approximately 140 μg/min per kg body wt; *3)* extracellular volume expansion produced by saline infusion, calcium infusion, and chronic treatment with deoxycorticosterone acetate (DCA) are each associated with a decrease in Tm for magnesium; and *4)* parathyroid hormone may directly enhance magnesium reabsorption.

METHODS

Thirty-two experiments were performed, either with or without pentobarbital anesthesia, in 30 female mongrel dogs weighing 12–27 kg. Respiration in the anesthe-

d dogs was controlled by a Harvard respirator pump a ventilation adjusted to maintain arterial pH within rmal range. Water diuresis was induced by oral water d of 500–800 ml. Glomerular filtration rate (GFR) s measured by exogenous creatinine clearance. Urine s collected from a retention catheter, and the bladder s washed with air at the end of each period to assure mpleteness of collection. Blood was withdrawn at the dpoint of each clearance period from an infying needle the left femoral vein. Magnesium chloride, added to 5% dextrose in water, was infused to deliver 1.0–3.0 g Mg/min per kg body wt over 3–6 hr. The following dies were performed:

1) *Magnesium chloride or sulfate infusion alone (eight dogs, 10 experiments)*. Four of the eight animals were trained and studied in the unanesthetized state. In all experiments, water diuresis was maintained by the infusion of 2.5% dextrose in water delivered at a rate equal to urine flow. In eight experiments, $MgCl_2$ was infused following three control clearance periods; in three of these studies, additional clearance periods were collected for 90 min after the $MgCl_2$ infusion was discontinued to evaluate renal handling of magnesium with a falling as well as a rising serum level. In an additional two experiments, an equivalent amount of magnesium was given in the form of $MgSO_4$ to investigate the influence of this nonreabsorbable anion.

2) *Simultaneous infusion of 0.9% NaCl and $MgCl_2$ (three anesthetized dogs)*. Normal saline was infused for 60 min at 30 ml/min and subsequently at a rate adjusted to urine flow. Three control clearance periods were obtained after the first 60 min of saline infusion, and $MgCl_2$ infusion was then superimposed throughout the remainder of the study.

3) *Simultaneous infusion of 0.9% NaCl, $CaCl_2$, and $MgCl_2$ (three anesthetized dogs)*. Normal saline was administered as in *study 2* above. After three control clearance periods, both $CaCl_2$ (10–20 mg Ca/kg per hr) and $MgCl_2$ were infused throughout the experiment.

4) *Simultaneous infusion of 0.9% NaCl and $MgCl_2$ in dogs pretreated with DCA (two anesthetized dogs)*. Each dog received intramuscular injections of 20 mg DCA daily for 7 days prior to the experiment, and an additional 10 mg DCA on the morning of the study. The protocol followed was identical to that described in *study 2* above.

In each experiment of the *studies 2, 3,* and *4* above, a triple-lumen catheter (U.S. Catheter and Instrument Co.) with a balloon attached to its distal end was inserted via the right femoral artery into the aorta to a level above the renal arteries. Abrupt inflation of the balloon invariably led to cessation of urine flow and confirmed its location. Intra-aortic pressure was monitored both proximal and distal to the balloon by mercury manometers. While serum magnesium was stable at a high level towards the end of each experiment, glomerular filtration rate was acutely reduced by inflation of the intra-aortic balloon. Five minutes were allowed for the intra-aortic pressure to stabilize, and three to six clearance periods of 5–7 min duration were then collected.

5) *Infusion of $MgCl_2$ in dogs pretreated with DCA (five anesthetized dogs)*. Each dog received DCA as described in *study 4* above. On the day of the study, three control periods were collected and then $MgCl_2$ was infused at 1.0–3.0 μg Mg/min per kg body wt for 6 hr.

6) *Simultaneous infusion of $CaCl_2$ and $MgCl_2$ (five anesthetized dogs)*. Calcium chloride, added to 2.5% dextrose in water, was infused to deliver 7 mg Ca/kg per hr throughout the entire experiment. After 60 min, three control periods were obtained and $MgCl_2$ was then infused for 6 additional hours.

7) *Parathyroid extract (Lilly) administration during $MgCl_2$ infusion (four anesthetized dogs)*. After three control clearance periods, $MgCl_2$ was infused for 6 hr. Two hundred units of parathyroid extract (PTE) were injected with the institution of $MgCl_2$ infusion and another 100 units of PTE was given every 2 hr thereafter. A total of 400 units were given.

Blood and urine samples were analyzed for creatinine, sodium, magnesium, and calcium by methods previously reported from this laboratory (19). Water content of serum was determined by refractometry (Goldberg refractometer, American Optical Co., Buffalo, N.Y.). Serum creatinine, sodium, calcium, and magnesium were measured in each clearance period. Diffusible calcium and magnesium were determined in ultrafiltrates of serum prepared anaerobically in the Lavietes chambers (17) at room temperature. Ultrafiltrates were prepared from specimens taken during one control period

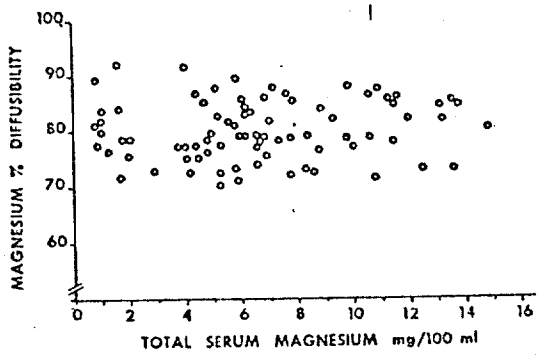


FIG. 1. Effect of variations in total serum magnesium on its percent diffusibility during magnesium infusion.

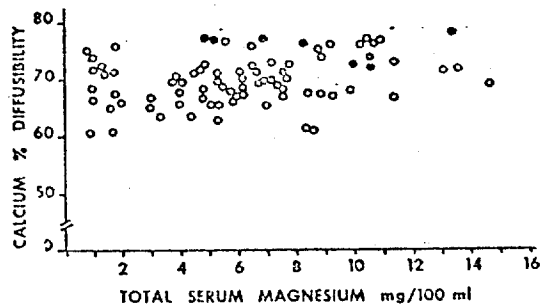


FIG. 2. Effect of variations in total serum magnesium on percent diffusibility of calcium with infusion of $MgCl_2$ alone (○), or $MgCl_2$ and $CaCl_2$ (●).

and every 90 min thereafter. Percent diffusibility of calcium and magnesium was calculated from the following formula:

$$\text{percent diffusibility} = \frac{\text{diffusible concentration (mg/100 ml serum)}}{\text{total concentration (mg/100 ml serum)}} \times 100$$

The percent diffusibility of both calcium and magnesium evidenced no appreciable change in any given experiment or in the total group with the various manipulations described (Figs. 1 and 2). Therefore, the mean percent diffusibility of calcium and magnesium in each experiment was used to calculate their diffusible fractions in those clearance periods where direct measurements were not made. The following formula was used:

$$\text{diffusible value} = \text{total concentration} \times \text{percent diffusibility}$$

All clearances of calcium and magnesium are expressed in terms of their diffusible fractions.

RESULTS

In the majority of experiments GFR remained relatively stable during the infusion of magnesium salts, although a fall in GFR of 10–30% occurred in 8 of 32 experiments when the blood levels of magnesium were highest. In both the anesthetized and unanesthetized dogs, magnesium infusion caused a rapid increase in magnesium excretion. The percent of filtered magnesium excreted ((magnesium clearance (C_{Mg})/creatinine clearance (C_{Cr})) × 100) rose steeply as diffusible serum magnesium was elevated from 1.5 to 7.0 mg/100 ml; with a further increase in the levels of diffusible serum magnesium (7.0–12.0 mg/100 ml), the fraction of filtered magnesium excreted increased much more slowly, reaching 60–75%. The relationship between (C_{Mg}/C_{Cr}) × 100 and the diffusible magnesium content of the serum was similar whether serum magnesium was rising or falling.

TABLE 1. *Detailed protocol of experiment in unanesthetized dog receiving $MgCl_2$ infusion*

Time, min	V, ml/min	C_{Cr}, ml/min	dSMg, mg/100 ml	$U_{Mg}V$, μg/min	$\frac{C_{Mg}}{C_{Cr}} \times 100$	dSCa, mg/100 ml	$U_{Ca}V$, μg/min	$\frac{C_{Ca}}{C_{Cr}} \times 100$	S_{Na}, mEq/liter	$U_{Na}V$ μEq/min	$\frac{C_{Na}}{C_{Cr}} \times 100$
0	Oral water load (300 ml)										
15	Priming dose of creatinine (800 mg)										
	Infusion I started: 10 mg creatinine/min delivered in 2.5% dextrose in water at 1 ml/min										
	Infusion II started: 2.5% dextrose in water infused at a rate equal to urine flow										
86–96	9.3	100.2	1.36	67	4.9	6.05	16	0.3	143	51	0.36
96–111	9.0	99.7	1.36	60	4.4	6.05	12	0.2	144	36	0.25
111–122	10.0	112.8	1.36	83	5.4	6.09	17	0.3	144	68	0.42
123	Infusion III started: $MgCl_2$ in 2.5% dextrose in water to deliver 1.0 μg Mg^{++}/kg per min at a rate of 1 ml/min										
123–134	9.7	105.4	1.59	194	11.6	5.96	29	0.5	143	59	0.39
134–144	10.2	106.8	2.08	447	20.1	5.83	60	1.0	143	68	0.45
144–154	10.0	97.3	2.48	595	24.7	5.80	95	1.7	142	66	0.43
154–164	9.4	91.7	2.93	741	27.5	5.80	112	2.1	142	56	0.43
164–174	9.3	97.4	3.23	944	30.0	5.78	136	2.4	141	58	0.42
174	Additional $MgCl_2$ added to infusion III to deliver 2.0 μg Mg^{++}/kg per min										
174–184	9.2	95.4	3.44	1,061	32.3	5.78	155	3.1	140	49	0.37
184–194	9.5	98.5	3.94	1,457	37.6	5.78	219	3.9	140	62	0.45
194–214	2.5	84.4	5.28	1,693	38.0	5.65	279	5.9	140	57	0.48
214–224	3.8	102.6	5.60	2,736	47.6	5.55	519	9.1	140	198	1.38
224–234	3.8	94.1	6.15	2,766	47.8	5.44	564	11.0	140	265	2.01
234	Additional $MgCl_2$ added to infusion III to deliver 3.0 μg Mg^{++}/kg per min.										
234–244	3.2	99.6	6.72	3,432	51.3	5.23	712	13.3	139	306	2.21
244–254	3.9	93.2	7.44	3,674	53.0	5.28	790	16.1	138	415	3.25
254–264	4.7	102.2	8.11	4,655	56.2	5.20	940	17.7	137	525	3.75
264–274	4.0	100.5	8.11	4,996	61.3	5.18	910	17.5	136	434	2.59
274	Infusion III ($MgCl_2$) discontinued										
274–284	5.0	106.9	7.70	4,615	56.1	5.11	938	17.2	136	558	3.83
284–294	4.8	102.7	7.12	3,843	48.2	5.11	744	14.2	136	496	3.55
294–304	7.0	114.5	6.66	3,850	50.5	5.13	807	13.7	136	440	2.83
304–314	5.8	113.3	5.93	3,206	47.7	5.10	717	12.4	136	395	2.52
314–324	6.8	108.2	5.95	2,967	46.1	5.07	620	11.3	136	336	2.23
324–334	7.1	100.0	5.57	2,151	38.6	5.04	418	8.3	136	178	1.31
334–344	8.6	99.3	4.91	2,175	44.6	4.98	344	6.9	136	119	0.88
344–354	10.4	122.9	4.40	2,525	46.7	4.99	293	4.8	136	91	0.54

Data are presented also during the falling serum magnesium following the discontinuation of $MgCl_2$ infusion. V = urine volume, C_{Cr} = creatinine clearance, dSMg = diffusible serum magnesium, $U_{Mg}V$ = magnesium excretion, C_{Mg} = clearance of diffusible magnesium, dS_{Ca} = diffusible serum calcium, $U_{Ca}V$ = calcium excretion, C_{Ca} = clearance of diffusible calcium, S_{Na} = serum sodium, $U_{Na}V$ = sodium excretion, and C_{Na} = sodium clearance.

e detailed protocol from one experiment in which $_2Cl_2$ was infused alone is given in Table 1, and the lts from all such experiments are presented in Fig. 3. The percent of filtered magnesium excreted during $_4SO_4$ infusion was not different from that observed h $MgCl_2$ infusion for any given level of diffusible um magnesium (Fig. 4).

With the rise in urinary magnesium, calcium excretion reased immediately (Table 1). Urinary calcium con- uted 15–20% of the filtered load when the diffusible els of serum magnesium reached 7.0–12.0 mg/100 ml. e change in the percent of filtered calcium excreted as um magnesium was elevated is shown in Fig. 5. dium excretion also increased with magnesium infu- n, but the increment was less pronounced than that of lcium and did not occur until 2 hr after the initiation the magnesium infusion (Table 1).

The simultaneous infusion of $MgCl_2$ and normal saline to normal and DCA-treated dogs and the infusion of $MgCl_2$, normal saline, and $CaCl_2$ to normal dogs resulted in the excretion of a greater percent of filtered magnesium for any given level of serum magnesium as compared to the data from $MgCl_2$ infusion alone. However, on no occasion did C_{Mg}/C_{Cr} exceed unity (Figs. 6 and 7).

The acute reduction of GFR (−41.1 ± 2.5%, mean ± SE) by the inflation of the intra-aortic balloon, when diffusible serum magnesium and the fractional excretion rates were highest, invariably resulted in a fall in the percent of filtered magnesium excreted. In seven of the eight experiments, absolute magnesium reabsorption remained relatively unchanged with the acute reduction in GFR. The data from all of these experiments are shown in Table 2.

The analysis of data from eight experiments with magnesium chloride infusion alone demonstrated a reabsorptive Tm for magnesium of approximately 140 μg/

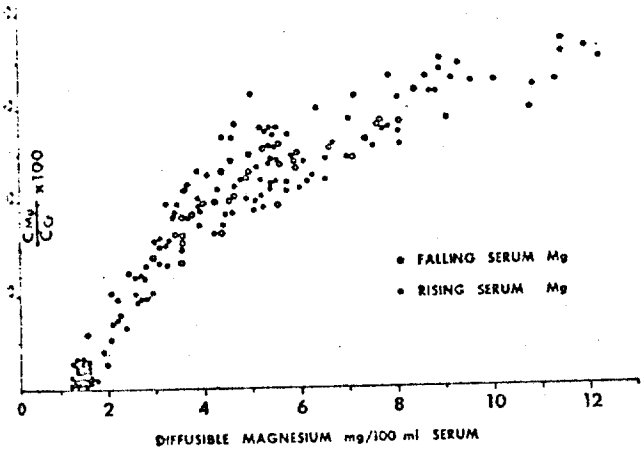


FIG. 3. Relationship between diffusible serum magnesium and ccent of filtered magnesium excreted during $MgCl_2$ infusion ne. C_{Mg} = clearance of diffusible magnesium and C_{Cr} = atinine clearance.

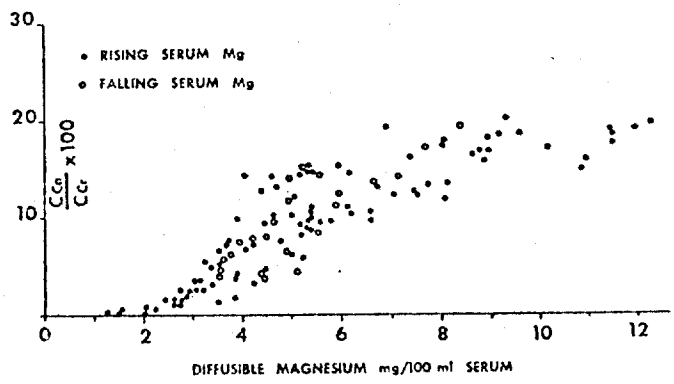


FIG. 5. Relationship between diffusible serum magnesium and percent of filtered calcium excreted during infusion of $MgCl_2$. C_{Ca} = clearance of diffusible calcium, C_{Cr} = creatinine clearance.

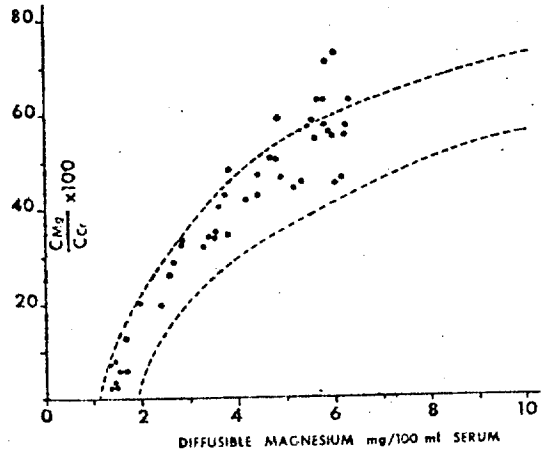


FIG. 4. Relationship between diffusible serum magnesium and rent of filtered magnesium excreted during $MgSO_4$ infusion. = clearance of diffusible magnesium and C_{Cr} = creatinine rance. Area between the dotted lines represents the relationship ved during $MgCl_2$ infusion.

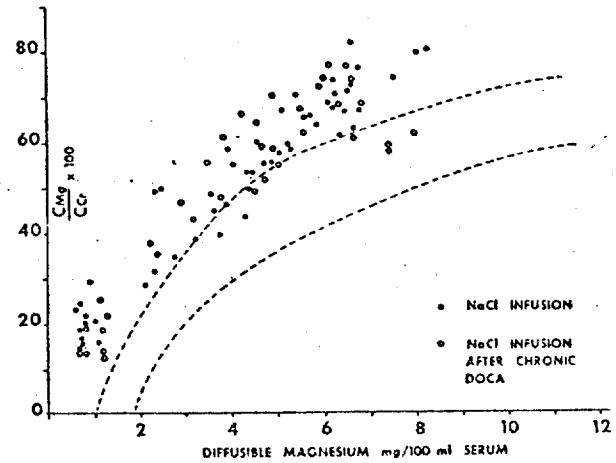


FIG. 6. Relationship between diffusible serum magnesium and percent of filtered magnesium excreted during infusion of 0.9% saline and $MgCl_2$ to normal and DCA-treated dogs. C_{Mg} = clearance of diffusible magnesium and C_{Cr} = creatinine clearance. Area between dotted lines represents relationship during infusion of $MgCl_2$ alone.

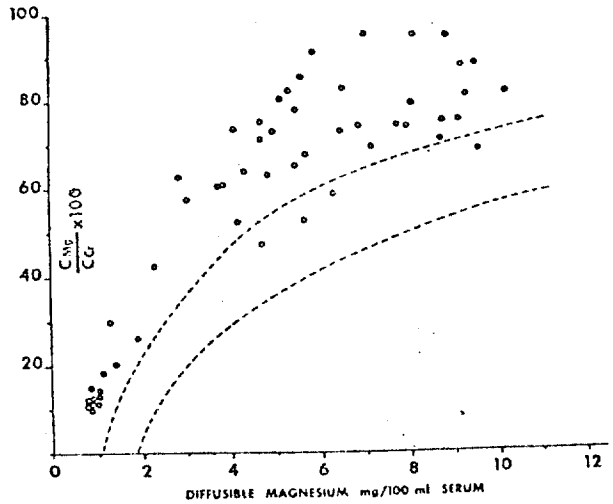


FIG. 7. Relationship between diffusible serum magnesium and percent of filtered magnesium excreted during infusion of 0.9% NaCl, $CaCl_2$, and $MgCl_2$. C_{Mg} = clearance of diffusible magnesium and C_{Cr} = creatinine clearance. The area between the dotted lines represents relationship obtained during $MgCl_2$ infusion alone.

TABLE 2. *Summary of all experiments with acute reduction of GFR by the inflation of the intra-aortic balloon*

Dog No.		V, ml/min	C_{Cr}, ml/min	dSMg, mg/100 ml	$U_{Mg}V$, μg/min	$\frac{C_{Mg}}{C_{Cr}} \times 100$	Mg Reabsorbed, μg/min
Normal dogs receiving 0.9% NaCl + $MgCl_2$							
1	Pre	7.4	33.8	6.62	1,586	70.4	638
	Post	2.6	23.9	6.50	687	44.9	867
2	Pre	7.6	39.1	7.60	2,357	79.1	615
	Post	3.6	25.5	8.83	1,660	73.5	592
3	Pre	6.2	74.8	5.97	3,011	67.3	1,455
	Post	2.9	47.3	6.79	1,907	58.7	1,305
Normal dogs receiving 0.9% NaCl + $CaCl_2$ + $MgCl_2$							
1	Pre	11.9	46.1	8.83	3,081	75.5	990
	Post	4.7	24.2	9.91	1,547	65.2	851
2	Pre	9.1	26.7	9.64	1,875	73.1	699
	Post	3.9	13.6	11.00	1,058	70.4	438
3	Pre	19.2	50.2	8.99	4,332	91.1	181
	Post	5.7	27.2	10.35	2,109	75.0	706
Doca-treated dogs receiving 0.9% NaCl + $MgCl_2$							
1	Pre	15.9	52.1	6.39	2,256	75.7	1,073
	Post	6.0	32.0	7.22	1,462	63.4	848
2	Pre	16.3	48.8	7.42	2,230	61.7	1,391
	Post	4.6	26.5	8.08	915	42.6	1,229

Pre = preinflation, post = post inflation. V = urine volume, C_{Cr} = creatinine clearance, dSMg = diffusible serum magnesium, $U_{Mg}V$ = magnesium excretion, C_{Mg} = clearance of diffusible magnesium, and DCA = deoxycorticosterone acetate. Each data point represents the mean of three to five clearance periods.

min per kg body wt. This Tm was reached when filtered magnesium was 280 μg/min per kg body wt (Fig. 8). There was a substantial decrease in Tm magnesium during saline or calcium infusion and following chronic

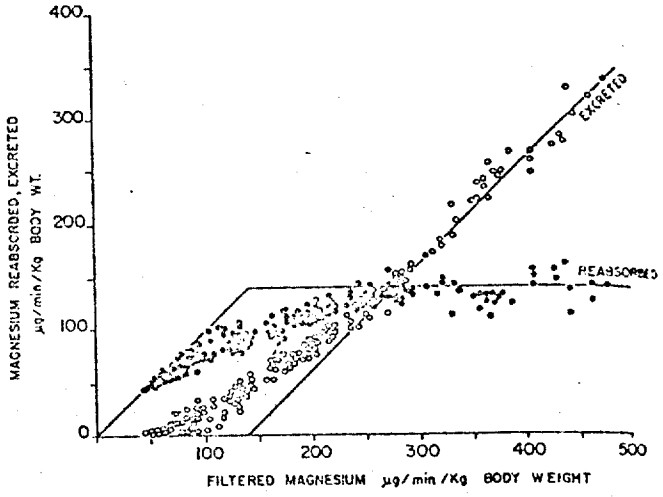


FIG. 8. Relationship between quantity of magnesium filtered through glomeruli and quantities reabsorbed by renal tubule and excreted in urine during $MgCl_2$ infusion. Data were obtained in 8 dogs and only in 6 animals filtered magnesium exceeded 280 μg/min per kg body wt and magnesium Tm was observed. Some data points at filtered levels of magnesium below 150 μg/min per kg body wt were omitted for clarity of illustration.

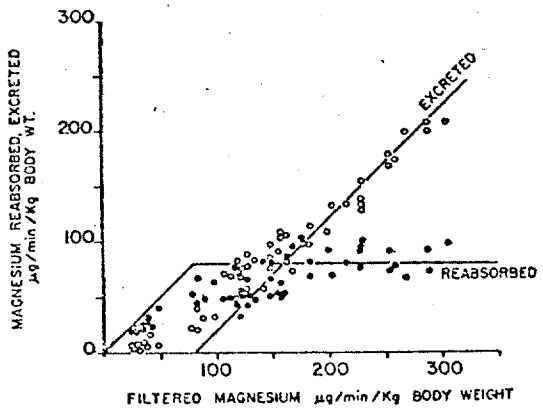


FIG. 9. Relationship between quantity of magnesium filtered through glomeruli and quantities reabsorbed by renal tubule and excreted in urine during the simultaneous infusion of $MgCl_2$ and NaCl. Data were obtained in 3 dogs.

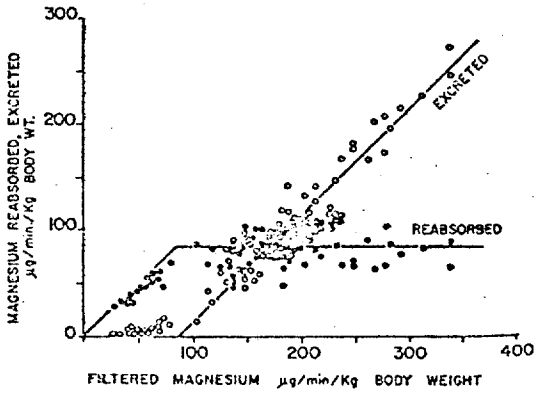


FIG. 10. Relationship between quantity of magnesium filtered through the glomeruli and quantities reabsorbed by renal tubule and excreted in urine during the simultaneous infusion of $MgCl_2$ and $CaCl_2$. Data were obtained in 5 dogs.

treatment with DCA as well (Figs. 9, 10, and 11); the Tm values were 80, 85, and 75 μg/min per kg body wt, respectively. Under these conditions the Tm values were reached at filtered loads of 150 μg/min per kg body wt.

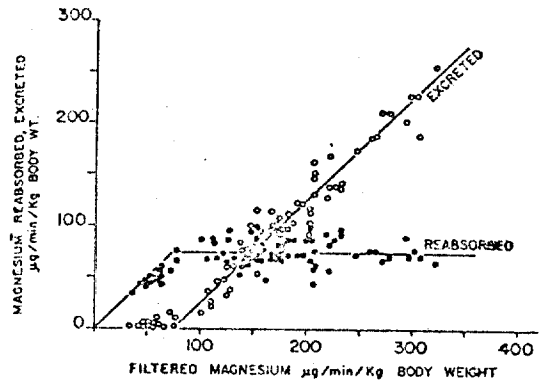


FIG. 11. Relationship between quantity of magnesium filtered through the glomeruli and quantities reabsorbed by renal tubule and excreted in urine during the infusion $MgCl_2$ in DCA-treated dogs. Data were obtained in 5 animals.

TABLE 3. *Detailed protocol of experiment with $MgCl_2$ infusion and parathyroid extract administration*

Time, min	C_{Cr}, ml/min	dSMg, mg/100 ml	$U_{Mg}V$, μg/min	$\frac{C_{Mg}}{C_{Cr}} \times 100$	dSCa, mg/100 ml	$U_{Ca}V$, μg/min	$\frac{C_{Ca}}{C_{Cr}} \times 100$
0	Oral water load (500 ml)						
15	Priming dose of creatinine (500 mg) Infusion I started: 8 mg creatinine/min delivered in 2.5% dextrose in water at 1 ml/min Infusion II started: 2.5% dextrose in water infused at a rate equal to urine flow						
115–135	50.9	1.43	39	5.4	6.68	7	0.2
135–155	53.9	1.44	44	5.7	6.87	7	0.2
155–175	47.6	1.50	41	5.7	6.92	9	0.3
175	$MgCl_2$ added to infusion I to deliver 1 μg Mg^{++}/kg per min Intramuscular injection of 200 units PTE						
175–205	56.6	2.20	139	11.2	6.81	17	0.4
205–235	63.5	2.91	366	19.8	6.71	52	1.2
235–265	53.6	3.53	617	32.6	6.86	113	3.2
265–295	50.4	4.46	891	37.7	6.66	188	5.6
295	Intramuscular injection of 100 units PTE						
295–325	48.8	4.92	988	41.2	6.86	274	8.2
325–355	45.2	5.44	1,147	46.6	7.06	277	8.7
355–385	47.0	5.76	1,417	52.3	7.39	344	9.9
385–415	46.9	6.42	1,496	49.7	7.70	318	8.8
415	Intramuscular injection of 100 units PTE						
415–445	47.0	6.74	1,770	55.9	7.96	392	10.5
445–475	49.8	7.55	1,656	44.0	8.25	439	10.7
475–505	49.9	8.29	1,494	36.2	8.35	392	9.4
505–535	47.1	8.28	1,656	42.5	8.45	423	10.7

C_{Cr} = creatinine clearance, dSMg = diffusible serum magnesium, $U_{Mg}V$ = magnesium excretion, C_{Mg} = clearance of diffusible magnesium, dSCa = diffusible serum calcium, $U_{Ca}V$ = calcium excretion, and C_{Ca} = clearance of diffusible calcium.

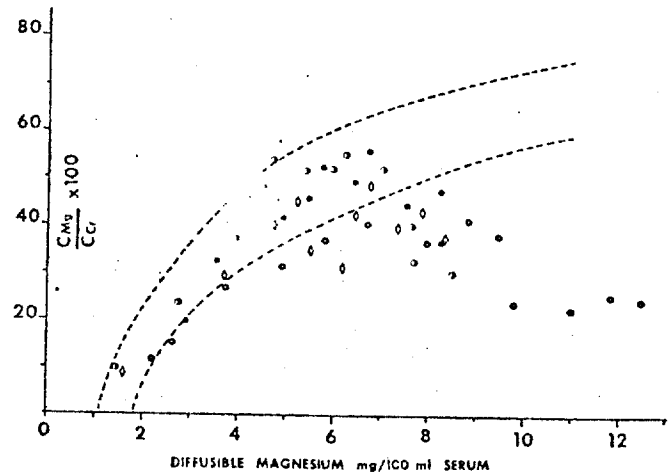


FIG. 12. Relationship between diffusible serum magnesium and the percent of filtered magnesium excreted during $MgCl_2$ infusion and PTE administration. C_{Mg} = clearance of diffusible magnesium and C_{Cr} = creatinine clearance. The area between the dotted lines represents the relationship obtained during $MgCl_2$ infusion. Each symbol represents a different experiment.

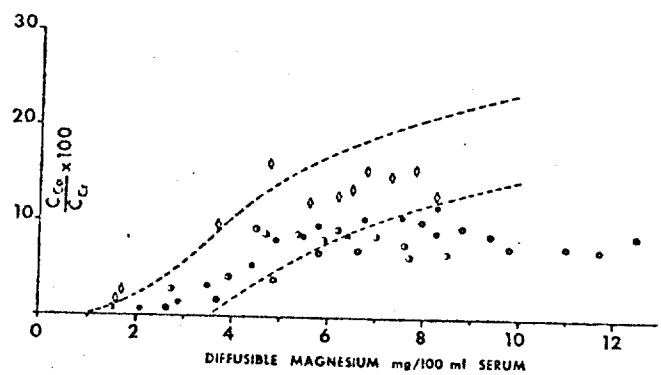


FIG. 13. Relationship between diffusible serum magnesium and percent of filtered calcium excreted during $MgCl_2$ infusion and PTE administration. C_{Ca} = clearance of diffusible calcium and C_{Cr} = creatinine clearance. Area between dotted lines represents relationship obtained during $MgCl_2$ infusion alone. Each symbol represents a different experiment.

Parathyroid extract administration during magnesium infusion caused a decrease in the percent of filtered magnesium excreted in all experiments as compared with results observed during $MgCl_2$ infusion alone. This effect became apparent 4 to 5 hr after the first injection of PTE. A detailed protocol of such an experiment is given in Table 3 and the data from all of these studies are depicted in Fig. 12. With PTE administration, urinary calcium was also lower when compared to results from experiments with $MgCl_2$ infusion alone (Fig. 13).

DISCUSSION

Although a maximum tubular reabsorptive capacity for magnesium was reported in rats (2) and chickens (21), data concerning such a Tm rate for magnesium reabsorption in dogs and humans are not definitive. The studies of Barker, Clark, and Elkinton in dogs (4) and of

Chesley and Tepper in pregnant women (9) suggested that the capacity of the renal tubule to reabsorb magnesium might be saturated at or near the normal plasma level. In their studies, direct measurements of diffusible serum magnesium were not made, and their tentative conclusion might have been based on an underestimation of the diffusible fraction of serum magnesium and its filtered load. In experiments by Knippers and Hehl (16) and by Samiy and co-workers (22), $MgCl_2$ was infused to dogs which were receiving variable quantities of saline. In neither studies were the data analyzed to determine the presence or absence of a Tm for magnesium. In the present study, when magnesium salts were infused alone, magnesium reabsorption increased two- to threefold as the filtered load of magnesium rose to 280 μg/min per kg body wt; above this level no further increase in magnesium reabsorption was seen (Fig. 8). The data demonstrate the presence of a maximum tubular reabsorptive capacity for magnesium of approximately 140 μg/min per kg body wt. This full capacity to reabsorb magnesium was not observed until the quantity filtered was approximately twice the Tm value. The marked degree of splay is consistent with a wide heterogeneity of nephron capacity for magnesium reabsorption.

During extracellular volume expansion produced by saline infusion, calcium infusion, or after chronic DCA treatment, the maximum tubular reabsorptive capacity for magnesium was 80, 85, and 75 μg/min per kg body wt, respectively. These observations indicate that these procedures were associated with a decrease in the Tm for magnesium. The data strongly suggest that the decrease in tubular reabsorption of magnesium observed at physiological serum magnesium levels and filtered loads during saline infusion, calcium infusion, or following chronic DCA treatment is probably the result of reduced Tm magnesium.

The observations that acute reduction in glomerular filtration rate had little or no effect on Tm magnesium (Table 2) indicate that the latter is independent of glomerular filtration rate. The tubular mechanism for reabsorption of magnesium may be saturated by a sufficient rise in plasma concentration and filtered load. Reduction in glomerular filtration rate would be expected to have no influence on maximum reabsorptive capacity as long as filtered load exceeds the saturation limit. A similar lack of dependence of maximal tubular reabsorption on glomerular filtration rate has been reported for other substances such as phosphate and glucose for which a maximum reabsorptive capacity exists (2, 23).

The lack of difference in the relationship between $(C_{Mg}/C_{Cr}) \times 100$ and the concentration of diffusible magnesium in serum while the latter was either rising during $MgCl_2$ infusion or falling after the infusion had been discontinued indicates that the change in filtered magnesium is the primary determinant of urinary excretion at these serum levels. Furthermore, these observations suggest that hormonal factors, which might be brought into play by hypermagnesemia, have little if any effect on renal handling of magnesium under these experimental conditions.

Observations that magnesium is secreted by the tubule of the aglomerular fish (7) have stimulated several investigators to evaluate whether this ion is also secreted by the mammalian nephron. Averill and Heaton (2) demonstrated magnesium secretion by the kidney of the rat following prolonged magnesium loading; other studies (1) did not confirm this observation. Available data regarding magnesium secretion by the dog kidney are equivocal. In a few clearance periods during the infusion of large quantities of magnesium to dogs, urinary magnesium exceeded filtered load (4, 16). Ginn and co-workers (12), utilizing stop-flow studies and radioactive magnesium, reported observations consistent with tubular secretion in the dog. However, their data do not necessarily indicate net secretion since passive transtubular exchange cannot be excluded. Other investigators have been unable to find evidence for renal secretion of magnesium in the dog (22). In the present study, magnesium excretion did not exceed filtered load during the infusion of large amounts of magnesium chloride or sulphate. Even when factors known to decrease tubular magnesium reabsorption such as NaCl infusion (8, 19), $CaCl_2$ infusion (10), and chronic DCA treatment (20) were superimposed on magnesium chloride infusion, the percent of filtered magnesium excreted did not exceed unity. However, if tubular secretion contributed to urinary magnesium during these procedures, one would expect that acute reduction in GFR and filtered load, while both serum magnesium and excretory rates are high, might unveil such a secretory process. Our data show that in every case the C_{Mg}/C_{Cr} fell with the reduction in GFR and filtered magnesium. It appears, therefore, that magnesium, like sodium and calcium, is handled by the kidney by filtration and tubular reabsorption alone. It should be pointed out that a secretory process of small magnitude, if at all present, might not be identified with the clearance technique. Such a theoretical secretory system, if quantitatively limited and quickly saturated, at or near the normal serum magnesium level, would be obscured as serum magnesium increases. The subsequent behavior of the renal handling of magnesium would appear to be entirely the result of filtration and reabsorption alone.

The intramuscular injection of parathyroid extract decreased fractional magnesium excretion in every animal despite the presence of high filtered magnesium which favors increased excretion. Fractional calcium excretion was also reduced raising the possibility that the change in urinary magnesium was secondary to that of calcium. This supposition seems remote. Since the augmentation of urinary calcium occurred secondary to magnesium loading and since the relative rates of magnesium reabsorption and excretion was so great, it is unlikely that changes in urinary calcium can influence magnesium excretion under these experimental conditions. It is more reasonable that both magnesium and calcium excretion were affected simultaneously by para-

thyroid extract. The present data, therefore, support the concept that parathyroid hormone can enhance tubular magnesium reabsorption per se.

This study was supported by Public Health Service Grants Am 07190 and Am 06087. A report was presented at the 1st Annual Meeting of the American Society of Nephrology, Los Angeles, Calif., 19 October 1967.

S. Massry is an Established Investigator of the American Heart Association.

Received for publication 20 May 1968.

REFERENCES

1. Alfredson, K. S., and M. Walser. Is magnesium secreted by the rat renal tubule? *Proc. Ann. Meeting Am. Soc. Nephrol., 2nd, Washington, D. C., 1968* p. 1.
2. Averill, C. M., and F. W. Heaton. The renal handling of magnesium. *Clin. Sci.* 31: 353–360, 1966.
3. Ayer, J. L., W. A. Schiess, and R. F. Pitts. Independence of phosphate reabsorption and glomerular filtration in the dog. *Am. J. Physiol.* 151: 168–173, 1947.
4. Barker, E. S., J. K. Clark, and J. R. Elkinton. Renal response to magnesium loading in the dog. *Federation Proc.* 16: 6, 1957.
5. Barker, E. S., J. R. Elkinton, and J. K. Clark. Studies of the renal excretion of magnesium in man. *J. Clin. Invest.* 38: 1733–1745, 1959.
6. Barnes, B. A., O. Cope, and T. Harrison. Magnesium conservation in the human being on a low magnesium diet. *J. Clin. Invest.* 37: 430–440, 1958.
7. Berglund, F., and R. P. Foster. Renal tubular transport of inorganic divalent ions by the aglomerular marine teleost, *Lophius americanus. J. Gen. Physiol.* 41: 429–440, 1958.
8. Brunette, M., S. F. Wen, and J. H. Dirks. Micropuncture study of magnesium reabsorption in the proximal tubule of the dog. *Clin. Res.* 16: 379, 1968.
9. Chesley, L. C., and I. Tepper. Some effects of magnesium loading upon renal excretion of magnesium and certain other electrolytes. *J. Clin. Invest.* 37: 1362–1372, 1958.
10. Coburn, J. W., S. G. Massry, L. W. Chapman, and C. R. Kleeman. The effect of sodium and calcium infusion on renal excretion of magnesium with normal and reduced filtered load (FMg). *Clin. Res.* 15: 354, 1967.
11. Fitzgerald, M. G., and P. Fourman. An experimental study of magnesium deficiency in man. *Clin. Sci.* 15: 635–647, 1956.
12. Ginn, H. E., W. O. Smith, J. F. Hammarsten, and D. Snyder. Renal tubular secretion of magnesium in dogs. *Proc. Soc. Exptl. Biol. Med.* 101: 691–692, 1959.
13. Heaton, F. W. The parathyroid glands and magnesium metabolism in the rat. *Clin. Sci.* 28: 543–553, 1965.
14. Heaton, F. W., and F. M. Parson. The metabolic effect of high magnesium intake. *Clin. Sci.* 21: 273–284, 1961.
15. Hirschfelder, A. D. Effect of renal insufficiency upon plasma magnesium and magnesium excretion after ingestion of magnesium sulphate. *J. Biol. Chem.* 104: 647–653, 1934.
16. Knippers, R., and U. Hehl. Die renale Ausscheidung von Magnesium, Calcium und Kalium nach Erhöhung der Magnesium-Konzentration im plasma des Hundes. *Z. Ges. Exptl. Med.* 139: 154–165, 1965.
17. Laviettes, P. H. Anaerobic ultrafiltration. *J. Biol. Chem.* 120: 267–275, 1937.
18. MacIntyre, I., S. Boss, and V. A. Troughton. Parathyroid hormone and magnesium homeostasis. *Nature* 198: 1958–1060, 1963.
19. Massry, S. G., J. W. Coburn, L. W. Chapman, and C. R. Kleeman. The effect of NaCl infusion on urinary Ca^{++} and Mg^{++} during reduction in their filtered loads. *Am. J. Physiol.* 213: 1218–1224, 1967.
20. Massry, S. G., J. W. Coburn, L. W. Chapman, and C. R. Kleeman. The effect of long-term desoxycorticosterone acetate administration on the renal excretion of calcium and magnesium. *J. Lab. Clin. Med.* 71: 212–219, 1968.
21. Robinson, R. R., and R. M. Portwood. Mechanism of magnesium excretion by the chicken. *Am. J. Physiol.* 202: 309–312, 1962.
22. Samiy, A. H. E., J. L. Brown, D. L. Globus, R. H. Kessler, and D. D. Thompson. Interrelation between renal transport system of magnesium and calcium. *Am. J. Physiol.* 198: 599–602, 1960.
23. Shannon, J. A., S. Farber, and L. Trodst. The measurement of glucose Tm in the normal dog. *Am. J. Physiol.* 133: 752–761, 1941.
24. Walser, M. Magnesium metabolism. *Ergeb. Physiol. Biol. Chem. Exptl. Pharmakol.* 59: 185–296, 1967.

Am. J. Physiol. 199:77

'limentary, blood and other changes due to feeding $MnSO_4$, $MgSO_4$ and Na_2SO_4[1]

JESSIE FISCHER MOINUDDIN AND HELEN WING-TSIT LEE
Department of Home Economics, Ohio Agricultural Experiment Station, Wooster, Ohio

MOINUDDIN, JESSIE FISCHER AND HELEN WING-TSIT LEE. *Alimentary, blood, and other changes due to feeding $MnSO_4$, $MgSO_4$ and Na_2SO_4.* Am. J. Physiol. 199(1): 77–83, 1960.—Rats were fed a basal diet or diets containing extra $MnSO_4$, $MgSO_4$ or Na_2SO_4 in three experiments. The major effects occurred in the experiment with the highest level of extra sulfates (138 mmoles/kg of feed) as follows. The $MgSO_4$-fed rats ate less feed than the Na_2SO_4-fed rats. Also, in the $MgSO_4$ group the activity of serum alkaline phosphatase was greater and the cleaned, dried cecum was considerably heavier than in the other groups, and the colon plus rectum was slightly longer than in the basal and $MnSO_4$ groups. The $MnSO_4$-fed rats, compared to rats of the other groups in this experiment, ate less feed, gained less body weight, required more feed per gram gain, drank more water, voided more urine, showed depigmentation of the labial surfaces of the incisor teeth, had less hemoglobin and serum inorganic phosphorus, and had cleaned, dried stomachs of lighter absolute weight. The erythrocyte count was higher in the $MnSO_4$ group than in the basal or Na_2SO_4 groups.

CERTAIN SUGARS IN THE DIET stimulate the growth of various regions of the gastrointestinal tract in rats. For example, diets containing 67% glucose or 67% sucrose, as compared to diets containing 67% cornstarch, increase the dry weight of the cleaned stomach (1). Diets containing 25% lactose (2) or 67% sucrose (1) increase the dry weight of the small intestine, and diets containing 15% cellobiose (1) or 10% arabinose (2) show some evidence of doing the same. Also, the 25% lactose (2), 10% arabinose (2) and 15% cellobiose (1) diets increase the dry weight of the cecum and of the colon plus rectum. These results, considered along with the number of sugar particles per unit weight of feed in each diet and with the digestibility or absorbability of each sugar, led to the idea that the increases produced in the growth of each organ by the designated sugars might be related to the osmotic effect of sugar particles in the lumen. However, results of feeding antibiotics indicated that microorganisms in the gut might play at least some part in some of the changes in gut weights. Thus, conceivably the sugars might be stimulating gastrointestinal growth in some way by serving as ready substrates for fermentation by the microflora of the lumen. Although we realized that there could be still other explanations of the stimulatory effects of dietary sugars, yet it seemed worthwhile to try to distinguish between osmotic effects of sugars and effects on microflora of the tract as possible causes of the stimulatory actions.

An indirect approach used in the present work was to see if gastrointestinal growth in the rat was increased by feeding substances (other than sugars) which have considerable osmotic effects in the lumen. For this purpose, we chose the hydragogue cathartics, magnesium sulfate and sodium sulfate. Manganese sulfate was tested also, since manganese has been found to stimulate the growth of the small intestine and of the ceca in chicks (3). It seemed unlikely that any of these three sulfates would affect the microflora of the lumen in the same way that the sugars might. However, some other effect of one or more of these salts on the microflora could not be ruled out. Also, there would be other uncertainties in the interpretation of any stimulation of growth by hydragogue cathartics. Thus, the present experiment was exploratory rather than a clear-cut test of alternatives. Other approaches to the problem are needed also.

METHODS

In three separate experiments, weanling male Sprague-Dawley rats were housed in individual cages and given tap water and their respective diets ad libitum for 4 weeks. In each experiment, there were six rats in each of the four dietary groups. In *experiments a* and *c*, feed intakes and feed:gain ratios were obtained for each week and for the total 4-week period. In all three experiments, records were kept of any diarrhea which occurred. The rats were weighed at the beginning of the 4-week period, at the end of each week, and at the end just before killing them. The rats were killed as described previously (2)

Received for publication January 22, 1960.
Approved for publication as journal article no. 91-59 by the State Director of the Ohio Agricultural Experiment Station.

#3839.

over a 3-day period. The gastrointestinal organs were freed from mesentery and excised. The small intestine and the colon plus rectum were hung full length, each with a 5-gram weight attached to the lower end. When these organs had straightened out completely, the lengths were measured. The organs were cleaned, examined, dried and weighed (2). In *experiment c*, a small snip of stomach was removed (for histological examination) before drying the organ, so that the dry weights of the stomach do not reflect the true weights as accurately as in *experiments a* and *b*.

In *experiment c*, additional observations were made as suggested by certain developments during the feeding period. These included visual observation of the teeth and of the volume of urine. Also, the amount of water drunk during the first 48 hours of the 3rd week was recorded. After 3½ weeks of the feeding period, blood was obtained from the tail for red and white blood cell counts, and for determination of hemoglobin by the oxyhemoglobin method. At the time of killing the rats, blood was collected from the severed great vessels in the neck, and the serum from this blood was analyzed for alkaline phosphatase (4), inorganic phosphate (5), and protein (6). Many tissues were preserved for later analysis and examination, but it has not yet been possible to arrange for completion of this part of the study.

The basal diet, designated as the cornstarch diet, in all three experiments contained 67% cornstarch (Buffalo brand no. 3401, generously donated by the Corn Products Sales Co., New York City), 24% 'vitamin-free' casein, 5% Crisco, 4% salt mixture (U.S.P. XIV), and vitamins as given in detail elsewhere (7). The salt mixture was purchased from the Nutritional Biochemicals Corp., and it contained 3.8% magnesium sulfate (anhydrous) and 0.02% manganous sulfate monohydrate, but no sodium sulfate. For the diets high in sulfates of manganese, magnesium and sodium, part of the cornstarch of the basal diet was replaced, respectively, by extra manganous sulfate (monohydrate, Mallinckrodt, analytical reagent, A.C.S.), extra magnesium sulfate (anhydrous powder, Mallinckrodt, analytical reagent), or sodium sulfate (anhydrous powder, Merck, A.C.S.). In *experiment a*, the amount of the respective sulfate salt added, over and above that in the salt mixture, for the $MnSO_4$, $MgSO_4$ and Na_2SO_4 groups was 0.88 mmole/kg. This level was fed throughout the experiment. In *experiment b*, the respective sulfates were added initially at a level (again, over and above that in the basal diet salt mixture) of 8.64 mmole/kg of feed. This level was progressively increased at the same times for the $MnSO_4$, $MgSO_4$ and Na_2SO_4 groups in *experiment b* by doubling it on the 9th day of the feeding period to 17.28 mmole/kg, doubling this on the 17th day to 34.56, doubling the latter on the 25th day to 69.12 and there maintaining the level for the remaining 4–6 days. The final percentage by weight in the diet of the major sulfate constituent (now including the contributions of the basal salt mixture) was 1.2% $MnSO_4 \cdot H_2O$, 1.0% $MgSO_4$ and 1.0% Na_2SO_4 for the respective high sulfate diets in *experiment b*. In *experiment c*, the extra sulfates were added at the level of 138 mmole/kg of feed throughout the experiment.

In *experiments b* and *c*, the numerical results were analyzed statistically by analysis of variance, the hypothesis in every case being that the groups were equal and the alternative that they were not equal. In those cases where the F value indicated that there was a difference among the groups at the 5% level or less, a multiple range test (8) was applied to determine which among the groups differed from any other. The results of the latter test are indicated in the tables for the 5% and 1% levels of probability, and are referred to as significant in text statements if $P < 0.05$.

RESULTS

No data are presented for *experiment a*, since, at the low levels of sulfate salt additions in this experiment, all quantities examined were similar in all groups. Also, there were no significant differences among the groups in body weight gains within *experiment b*, so these gain data also are omitted.

Gains in body weight, intakes of feed and water, and feed : gain ratios (table 1). In *experiment c*, the rats in the $MnSO_4$ group, as compared to the rats in the other groups, gained less weight, ate less feed, required more feed per gram of gain and drank much more water. Although the $MgSO_4$-fed rats suffered chronic diarrhea (see later), they did not drink more water than the rats fed the cornstarch or Na_2SO_4 diets. The $MgSO_4$-fed rats ate significantly less feed than the Na_2SO_4-fed rats; they tended to eat less feed than the cornstarch diet rats also, but this latter tendency was significant only in the 1st week of the experiment. Thus $MgSO_4$ resembles another magnesium salt, $MgCl_2$, which also depresses the intake of feed when fed at excessive levels (9).

Volume of urine. In *experiment c*, rats in the $MnSO_4$ group voided more urine than rats in the other groups, as shown by much larger pools of urine on the waxed papers placed beneath the rats. The increase in volume

TABLE 1. *Gains in Body Weight, Intakes of Feed and Water, and Feed: Gain Ratios in Experiment c*

Dietary Group	Gain in 4 Wk.	Feed Intake in 4 Wk.	Over-All Feed: Gain Ratio for 4 Wk.	Water Intake, 1st 48 Hr. of 3rd Wk.
	gm	*gm*	*gm/gm*	*ml*
Cornstarch	186 (178–199)	386 (375–396)	2.08 (1.97–2.16)	48 (36–58)
$MnSO_4$	109* (101–124)	301* (280–319)	2.76* (2.52–2.89)	83* (53–99)
$MgSO_4$	180 (162–195)	356† (324–392)	1.98 (1.84–2.12)	48 (37–55)
Na_2SO_4	192 (180–209)	408 (371–453)	2.13 (2.03–2.19)	51 (40–60)

Figures given are averages; ranges are in parentheses. Average b. wt. at start was 55 gm in each of the groups. * Significantly different from the other 3 groups ($P < 0.01$). † Significantly different from the Na_2SO_4 group ($0.01 < P < 0.05$).

was estimated as at least twofold, but exact measurements were not made.

Teeth. During the 3rd week of *experiment c*, we noticed that the front surfaces of the incisor teeth of five out of six rats in the $MnSO_4$ group were white or very pale yellow in color. Only one rat in the $MnSO_4$ group had teeth of the normal orange-yellow color, whereas every rat in each of the other groups had normally colored incisors. These differences persisted until the end of the experiment.

Blood findings in experiment c (*table 2*). Hemoglobin in the $MnSO_4$ group was significantly lower than in the other three groups. Only one rat in the $MnSO_4$ group had a hemoglobin content falling within the range of the other three groups; this was the same rat which had normally colored incisor teeth. The numbers of red blood cells were significantly greater in the $MnSO_4$ group than in the cornstarch or Na_2SO_4 groups. The differences among the groups in numbers of white blood cells were not significant statistically. The differences in serum protein were not significant; however, three values in the $MnSO_4$ group were slightly below the normal range of 6.5-7.1 gm./100 ml (10). The $MgSO_4$ group exceeded the other groups in serum alkaline phosphatase activity. Serum inorganic phosphate was lower in the $MnSO_4$ group than in the other groups, in agreement with some other work in which fairly high levels of manganese were fed to growing rats (11, 12).

Diarrhea. In *experiment b*, there were no cases of diarrhea in the cornstarch group, one slight case in the $MnSO_4$ group and two slight cases in the Na_2SO_4 group lasting only 1 day each. In the $MgSO_4$ group, two of the six rats had no diarrhea, three had only slight diarrhea for 1 day, another on 2 nonsuccessive days, and the other on 3 nonsuccessive days), while the remaining rat had slight diarrhea on 6 different days (including 4 in succession) during the middle periods of the experiment and moderate diarrhea for the 3 successive days following the first increase in $MgSO_4$ level. Also, on certain other days when diarrhea did not occur, the feces of this rat and of one other rat in the $MgSO_4$ group were soft and had a variety of unusual shapes: some pellets were extra long but of normal thickness, some were very thin but of normal length, and some showed the lines of 'working over' plainly. Also, several of the pellets had several small sideways projections at one end and thus resembled cloves. The pellets were black in all groups.

In *experiment c*, there was no diarrhea in the $MnSO_4$ group and only one instance of slight diarrhea lasting 1 day in the cornstarch group. Only one rat in the Na_2SO_4 group had any diarrhea at all. This occurred on 4 different days (including 3 in succession) in about the middle of the feeding period, and was slight in each instance. Diarrhea began in all the rats of the $MgSO_4$ group after 1-3 days of commencing the feeding, and continued until the end of the experiment. Once established, diarrhea in this group was severe on most days during the first 3 weeks, and slackened in severity slightly in some of the rats during the 4th week. However, there was no clear-cut indication of remission of the $MgSO_4$-produced diarrhea, such as occurs with the diarrhea produced by various sugar-containing diets (1, 2, 7). Haag and Palmer (13) also found that marked catharsis continued throughout many weeks of an experiment when rats were fed diets containing 0.8-0.9% magnesium as either the sulfate or the carbonate and amounts of calcium and phosphorus similar to those in the diets herein.

Appearance of organs. In *experiment b*, the cecal contents in the $MgSO_4$ group were quite runny (although the liquid contained some solid material) as compared to the more pasty or waxy cecal contents in the other groups. In the $MgSO_4$ group of *experiment c*, the contents of the cecum and colon were runny, and the cecum was noticeably enlarged compared to the cecum in the other groups. The tissues around the rectum in the $MgSO_4$ group seemed to be tougher and more difficult to separate from the rectum than is usual, perhaps indicating that some adaptation occurred external to the organ itself.

Weights and lengths of organs (*table 3*). In *experiment b*,

TABLE 2. *Blood and Serum in Experiment c*

Dietary Group	Tail Blood After 3½ Weeks of Feeding the Diets			Serum From Blood From Vessels in Neck, Obtained at Killing		
	Hemoglobin	Red blood cells	White blood cells	Protein	Alkaline phosphatase	Inorganic phosphate
	gm./100 ml	*millions/cu. mm.*	*thousands/cu. mm.*	*gm./100 ml*	*Bodansky U./100 ml*	*mg. P/100 ml*
Cornstarch	14.4 (13.2-16.3)	6.91 (6.00-7.60)	9.19 (4.75-15.45)	6.7 (6.4-6.9)	84 (73-91)	5.3 (4.4-6.2)
$MnSO_4$	11.6* (10.0-14.0)	8.80† (7.40-11.00)	16.21 (11.95-21.20)	6.3 (5.7-7.0)	85 (81-89)	3.8§ (3.6-3.8)
$MgSO_4$	14.6 (14.1-15.4)	7.74 (6.90-8.75)	12.23 (6.70-16.60)	6.8 (6.6-7.0)	99‡ (90-105)	6.0 (4.8-7.0)
Na_2SO_4	14.6 (14.0-15.2)	6.94 (5.85-8.05)	11.72 (2.20-23.40)	6.5 (6.3-6.8)	78 (66-83)	5.3 (4.8-5.8)

Values given are averages; ranges are in parentheses. Six rats per group, except that the values and statistical analyses for phosphatase and phosphate are for 4 of the 6 rats in each group. The determinations of phosphatase in the sera of the other 2 rats of each group were ruined. The pooled sera of these remaining 2 rats of each group yielded values of 6.1, 3.7, 6.3 and 5.8 mg. P/100 ml for cornstarch, $MnSO_4$, $MgSO_4$, and Na_2SO_4 groups, respectively. * Significantly different from the other 3 groups ($P < 0.01$). † Significantly different from cornstarch and Na_2SO_4 ($0.01 < P < 0.05$). ‡ Significantly different from Na_2SO_4 ($P < 0.01$) and from cornstarch and $MnSO_4$ ($0.01 < P < 0.05$). § Significantly different from $MgSO_4$ ($P < 0.01$) and from cornstarch and Na_2SO_4 ($0.01 < P < 0.05$).

TABLE 3. *Body Weight, Weights of Cleaned, Dried Organs and Length of Small Intestine and Colon Plus Rectum in Experiments b and c*

Dietary Group	B. Wt.	Weights of Cleaned, Dried Organs				Lengths of Organs	
		Stomach	Small int.	Cecum	Colon+rectum	Small int.	Colon+rectum
	gm	*mg*	*mg*	*mg*	*mg*	*cm*	*cm*
				Experiment b			
Cornstarch	211 (193–235)	214 (196–235)	945 (877–966)	65 (52–74)	144 (130–164)	99 (96–105)	17.2 (16.5–18.5)
$MnSO_4$	210 (187–234)	231 (211–243)	904 (760–993)	73 (64–84)	150 (133–163)	100 (95–112)	17.6 (16.5–19.5)
$MgSO_4$	216 (171–237)	222 (184–244)	922 (844–1007)	80* (63–90)	145 (124–165)	98 (91–103)	17.8 (17.0–18.5)
Na_2SO_4	211 (172–246)	218 (189–247)	875 (798–1029)	63 (51–75)	142 (125–162)	96 (87–101)	17.1 (15.5–18.5)
				Experiment c			
Cornstarch	248 (232–260)	222 (202–238)	1011 (831–1174)	82 (70–97)	162 (145–184)	104 (99–107)	17.4 (16.5–19.0)
$MnSO_4$	171† (156–190)	179‡ (139–210)	915 (828–1025)	68 (60–75)	157 (141–180)	101 (94–105)	17.4 (15.5–19.0)
$MgSO_4$	243 (228–260)	217 (198–236)	1029 (961–1116)	161† (140–183)	165 (147–201)	105 (98–111)	19.7§ (18.5–21.0)
Na_2SO_4	256 (236–281)	229 (213–244)	1047 (1000–1134)	80 (71–90)	156 (144–167)	107 (104–112)	18.0 (16.0–19.5)

Values given are averages; ranges are in parentheses. Various groups are compared only within each experiment. In *experiment c*, weight of stomach is low in all groups due to removal of a small sample for histological examination. In *experiment c*, weights of small intestine were found to be different by analysis of variance ($0.01 < P < 0.05$), but no differences between single means were found using the multiple range test. * Significantly different from Na_2SO_4 and cornstarch ($0.01 < P < 0.05$). † Significantly different from the other 3 groups ($P < 0.01$). ‡ Significantly different from cornstarch and Na_2SO_4 ($P < 0.01$), and from $MgSO_4$ ($0.01 < P < 0.05$). § Significantly different from cornstarch and $MnSO_4$ ($0.01 < P < 0.05$).

the cecum was significantly heavier in the $MgSO_4$ group than in the cornstarch and Na_2SO_4 groups, but the differences were not very large.

In *experiment c*, the $MnSO_4$ group was exceeded by the other three groups in final body weight and in the absolute weight of the stomach. The absolute weight of the small intestine also tended to be least in the $MnSO_4$ group. However, the $MnSO_4$ group significantly exceeded the other three groups in the weight of the stomach and small intestine in relation to body weight (not given in table 3). The $MgSO_4$ group exceeded the other groups in the absolute and relative weights of the cecum. $MnSO_4$ exceeded Na_2SO_4 in relative cecal weight (not given in table 3). The absolute weights of the colon plus rectum did not differ significantly among the groups, but the relative weights of this organ (not given) were significantly greater in the $MnSO_4$ group than in the other groups. Most of the differences in weights of organs in *experiment c* appear to be related to the smaller size and body weight attained by the rats in the $MnSO_4$ group; there are difficulties in interpreting differences in weights of organs in such a situation. Thus, while the absolute weights of the small intestine, the cecum and the colon plus rectum in the $MnSO_4$ group were lowered less drastically than was the body weight, we don't know if the growth of these intestinal organs is stimulated by $MnSO_4$ or if it simply is not poisoned as greatly by $MnSO_4$ as is the growth of the body in general. The only clear-cut, interpretable effect of diet upon weights of organs in *experiment c* is the large increase in the weight of the cecum in the $MgSO_4$ group.

In *experiment c*, the colon plus rectum was longer in the $MgSO_4$ group than in the cornstarch or $MnSO_4$ groups. The ratio of weight to length of this organ (not given in table 3), like the absolute weight, did not differ significantly among the groups. Many other dietary substances (agar, for example) increase the colonic weight and weight:length ratio, as well as the length, in relation to the cornstarch group (14). Magnesium sulfate is the first substance we have found, among those which have any effect at all on the colon plus rectum, which appears to increase only the length.

DISCUSSION

Effects of $MnSO_4$. Most of the significant differences among the groups at the highest level of sulfates fed (that is, in *experiment c*) were due to the $MnSO_4$ group differing from the other three groups. These differences probably were due to manganese rather than to sulfate, since the $MgSO_4$ and Na_2SO_4 diets contained the same amounts of sulfate as did the $MnSO_4$ diet, and since more of the $MgSO_4$ and Na_2SO_4 diets were eaten. However, it remains possible that sulfate may affect the interaction of manganese and other elements.

We found that manganese, at the highest level we fed, caused decreases in growth, in intake of feed and in efficiency of conversion of feed to gain in body weight. Others have also reported that manganese in excess depresses the growth of young rats (11, 12, 15–17). Several changes possibly may be involved separately in causing this slow growth. First, an increased loss in the feces of phosphorus resulted from feeding excessive amounts of manganese (11), and low levels of inorganic phosphorus were found in the serum of manganese-fed young rats when the diets contained about 0.25% (12), 0.36% (11) or 0.47% (present paper) of phosphorus. Raising the dietary level of phosphorus to 0.80% raised the level of serum inorganic phosphorus and markedly improved growth (11). Second, the slowness of growth may have been related, in part, to the anemia caused by excessive manganese (17, present paper). Third, depletion of the rat's bodily thiamine by manganese and fourth, some alteration of energy metabolism mediated through the

thyroid gland are possible factors suggested by the work of others but regarding which we have no information from the present experiments.

Increases in the intake of water and in the volume of urine appear not to have been reported previously as symptoms of toxicity of manganese. In regard to possible causes of these increases, it may be pertinent that a somewhat prolonged decrease in the level of serum magnesium occurred in dairy calves fed supplemental manganese in experimental diets (18), and that a temporary decrease in serum magnesium occurred in cows grazing pastures of high manganese content and in rabbits, sheep or cows given manganese salts (19). Also, the volume of urine increased during magnesium deficiency in the rat (10). So it seems possible that excessive manganese increased the volume of urine by interfering with the absorption or metabolism of magnesium, thus inducing a deficiency of magnesium. Or possibly, since excessive manganese disturbs the iron picture (see later), the diuretic effect of $MnSO_4$ may involve interference with ferritin, the iron-storage protein which has been shown also to have an antidiuretic action (20). (This latter possibility is rendered somewhat less likely by the finding that apoferritin, the iron-free portion of ferritin, also is antidiuretic (20)). Other ways in which $MnSO_4$ might have produced the thirst and diuresis are conceivable and are not ruled out. Whether the increased intake of water may have caused the diuresis or vice versa is not known.

The depigmentation of the incisor teeth we observed in manganese-poisoned rats may have resulted directly from lowered iron, since rats given an excess of manganese have lower concentrations of plasma iron and lower stores of total body iron (17) and since it is known that simple iron deficiency causes loss of the yellow iron-containing pigment (21). Or, the manganese-produced depigmentation may have been secondary to a conditioned deficiency of phosphorus, since we found (herein) low values for serum phosphorus and since a diet low in phosphorus produced some bleaching of the yellow pigment (22). Hypoplasia of the enamel of the rat's upper incisor has been noted histologically after subcutaneous injection of manganese chloride, but it was not mentioned whether grossly apparent depigmentation, as seen in our manganese-fed rats, occurred also (23).

The present observations and some other work with several species indicate that an excess of manganese may notably affect the levels of hemoglobin and numbers of erythrocytes. Some of this work has been reviewed in various places (see, for example, 24). A decrease in hemoglobin and an increase in the red blood cell count, as reported in the present work, have been found in various species, although other effects or no changes also have been found. One per cent of manganese added to a stock diet for normal rats decreased hemoglobin but had no effect on the number of red cells (25). A combination of excessive oral and injected manganese in rats decreased hemoglobin (17) and, from the data presented, appeared to increase the numbers of red blood cells somewhat, although the increase in red cells is not indicated as being significant (17). Thus in the rat, lowering of hemoglobin by excessive manganese seems to be better established than is an increase in red blood cells. Also, excessive manganese promoted reticulocytosis in rats (17). Manganese in excess markedly affects both the iron picture (17, 26) and the copper picture (17, 26) in the body. However, whether manganese may produce or prolong anemia primarily by antagonism of iron or of something else (e.g. copper) is not known. Iron might appear to be involved mainly, since anemia was reversed by iron in manganese-fed pigs (27) but not by copper in rats given manganese (17).

Alkaline phosphatase of serum. Magnesium activates the alkaline phosphatase of rat serum (28, 29). The activity of this enzyme in rat serum is decreased when magnesium is deficient (30) and is either increased (31) or unchanged (32) when manganese is deficient in the diet. Thus it was of interest to learn the effects of dietary excesses of these metals on the activity, especially in view of the depression of serum magnesium by an excess of manganese in several mammalian species (18, 19), and also in view of the knowledge that magnesium and manganese may be at least partially interchangeable *in vitro* as activators of mammalian tissue alkaline phosphatases (33) and other enzymes. Our finding that the activity of serum alkaline phosphatase in the $MnSO_4$ group did not differ significantly from that in the cornstarch group may indicate that manganese was not specifically interfering with magnesium in our experiment, at least at the time we tested the enzymatic activity. However, it might also be that manganese did disturb the metabolism of magnesium in one or more other ways, but that manganese either did not displace magnesium from this particular enzyme or else was able to replace magnesium functionally as the activator of this enzyme *in vitro*. The slight but significant increase in the activity in the $MgSO_4$ group may indicate that the circulating enzyme(s) was not fully activated by magnesium under the conditions of the other diets. (Rosenthal *et al.* (29) found that plasmatic alkaline phosphatase of the rat was only about 75% as active in the absence as in the presence of added magnesium in the assay system, in a variety of nutritional states. We did not add any magnesium to our assay system.) It also seems possible that the extra magnesium of the $MgSO_4$ diet induced the formation and release (or simply the release) into the serum of more alkaline phosphatase, but we have no data on this point.

Gastrointestinal tract. An increase in the amount of manganese, added as manganese sulfate, from 2.6 ppm to about 50 ppm in the diet in *experiment a* had no effect on the weight of the cecum or small gut in our rats. This contrasts with the finding that the ceca and small gut of the chick grow heavier when the diet contains 50 ppm manganese as compared to 10 ppm manganese (3). The very much larger amounts of manganese in the high-manganese diet of our *experiment b* produced only a slight (statistically nonsignificant at the 5% level)

increase in cecal weight. Thus, it appears that the rat's cecum and small gut are either much less susceptible than the chick's to stimulation by dietary manganese, or else that other conditions in our experiments were too different from those in the experiment with chicks to be able to demonstrate a similar effect. The toxicity of manganese, particularly as reflected in poor growth, in *experiment c* prevents reaching any conclusion about effects on the rat's gastrointestinal tract of still higher levels of manganese.

Diarrhea and cecal distention, such as we noted herein with excessive $MgSO_4$ in *experiment c*, occur also with excessive dietary $MgCl_2$ or $MgCO_3$ (9). The weight of the cecum plus its contents was greatly increased by $MgCl_2$ or $MgCO_3$ (9); however, the weight of the cecal tissue alone was not given (9). Our findings with $MgSO_4$ show that the distention is accompanied by an actual increase in cecal tissue, not just by a stretching of the wall.

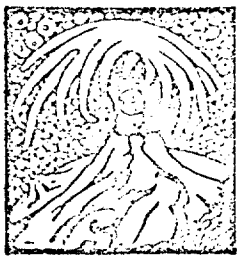
Many factors need to be considered before finally judging the possible usefulness of the hydragogue hypothesis to explain sulfate-produced increases in growth of any gastrointestinal region. Among these factors are the calculated numbers of extra particles per 100 gm feed which are supplied by the extra sulfate salts. These numbers for *experiment c*, assuming 100% ionization in the digestive tract, are 0.166×10^{23} for $MnSO_4$ and for $MgSO_4$, and 0.249×10^{23} for Na_2SO_4. (For comparison, the numbers of sucrose or glucose particles per 100 gm feed in previous experiments (1) were: 67% sucrose diet, 1.179×10^{23}; 25% sucrose diet, 0.440×10^{23}; 67% glucose (hydrate) diet, 2.037×10^{23}; and 25% glucose (hydrate) diet, 0.760×10^{23}. In Friedman's 50% talc diet (34), feeding of which stimulates growth of the small intestine in mice, perhaps by a hydragogue action, there are 0.794×10^{23} talc molecules per 100 gm of feed; the number of particles (molecules plus ions) from this talc probably would be somewhat greater than this, depending upon the degree of ionization in the lumen, which is unknown.) In addition to the number of particles per unit weight of feed, many other factors may affect the intensity and duration of a hydragogue action. Obviously, the total amount of feed eaten, and the amount eaten at any one meal, will influence the number of extra particles present in the tract. Other factors are extent of coprophagy, rate and completeness of digestion and absorption, rate of gastric emptying and intestinal transit, rate of excretion of absorbed substances in the bile or through the intestinal wall, and disposition by microbial action. If we consider only the number of small particles furnished by the diets, the present group of exploratory experiments does not adequately test the idea that a hydragogue action of certain substances is involved in their stimulation of organal growth. To cite just one example, in the stomach immediately after eating a similar weight of different diets (when very little absorption or emptying might have occurred), the number of extra small particles furnished by the diets high in sulfate salts is considerably less than the number of sugar particles furnished by the 67% glucose and 67% sucrose diets. The latter two diets are the only ones we have found which increase the gastric weight (1). Furthermore, the number of particles from the sulfate salts is smaller also than the number of sugar particles from 25% glucose and 25% sucrose diets, which diets fail to increase the gastric weight (1). So perhaps one might hardly have expected to have found any effect of our high sulfate diets on gastric weight, if a hydragogue effect is indeed involved in stimulation of gastric growth by the diets high in sugars, assuming that other factors are equal for the sulfate and sugar diets. However, as mentioned above, we cannot assume that these other factors are equal for all substances. Thus, differences in rate of gastric emptying could quickly change the concentration of different substances, and so alter the osmotic effect. Similar considerations apply to other factors and other regions of the tract. Until the many other factors are investigated thoroughly, the usefulness in gastrointestinal physiology of the present group of exploratory experiments is twofold. First, we have learned that feeding excessive amounts of $MgSO_4$ increases the weight of cecal tissue and appears to lengthen the colon plus rectum. Second, we have obtained some idea of the levels of sulfate salts which can be fed without untoward effects. Thus, in *experiment c*, the toxic effects of manganese sulfate and the digestive disturbances due to magnesium sulfate suggest that we could not sensibly raise the dietary levels of these two salts any higher, if it were necessary to do so to more adequately test the hydragogue theory of gastrointestinal growth stimulation. However, higher levels of sodium sulfate could be tested.

We are indebted to Mrs. Doris Elliot and Miss Margaret Thornbury for help with *experiments a* and *b*, respectively, to Mr. Robert Schlitz for the blood cell counts in *experiment c*, and to Dr. M. Moinuddin for help with the assays of *experiment c*.

REFERENCES

1. Moinuddin, Jessie F. and Helen W.-T. Lee. *Am. J. Physiol.* 192: 417, 1958.
2. Fischer, Jessie E. *Am. J. Physiol.* 188: 550, 1957.
3. Pepper, W. F., S. J. Slinger and I. Motzok. *Poultry Sc.* 32: 656, 1953.
4. Bodansky, A. *J. Biol. Chem.* 101: 93, 1933.
5. Fiske, C. H. and Y. Subbarow. *J. Biol. Chem.* 66: 375, 1925.
6. Kingsley, G. R. *J. Lab. & Clin. Med.* 27: 840, 1942.
7. Fischer, Jessie E. *Am. J. Physiol.* 188: 49, 1957.
8. Duncan, D. B. *Biometrics* 11: 1, 1955.
9. Elmslie, W. P. and H. Steenbock. *J. Biol. Chem.* 82: 611, 1929.
10. Greenberg, D. M., S. P. Lucia and E. V. Tufts. *Am. J. Physiol.* 121: 424, 1938.
11. Chornock, C., N. B. Guerrant and R. A. Dutcher. *J. Nutrition* 23: 445, 1942.
12. Blumberg, H., D. H. Shelling and D. A. Jackson. *J. Nutrition* 16: 317, 1938.

13. Hasg, J. R. and L. S. Palmer. *J. Biol. Chem.* 76: 367, 1928.
14. Mohiuddin, Jessie F. and Helen W.-T. Lee. *Am. J. Physiol.* 167: 693, 1959.
McCarrison, R. *Indian J. M. Res.* 14: 641, 1927.
Becker, J. E. and E. V. McCollum. *Proc. Soc. Exper. Biol. & Med.* 38: 740, 1938.
17. Gerber, C. J., D. S. Taylor, E. J. Eichwald, G. E. Cartwright and M. M. Wintrobe. *Proc. Soc. Exper. Biol. & Med.* 86: 223, 1954.
18. Hawkins, G. E., Jr., G. H. Wise, G. Matrone, R. K. Waugh and W. L. Lott. *J. Dairy Sc.* 38: 536, 1955.
19. Blakemore, F., J. A. Nicholson and J. Stewart. *Veterinary Rec.* 49: 415, 1937.
20. Baez, S., A. Mazur and E. Shorr. *Am. J. Physiol.* 162: 198, 1950.
21. Ratner, S. *J. Dental Res.* 15: 89, 1935.
22. Smith, M. C. *J. Dental Res.* 15: 281, 1936.
23. Wessinger, G. D. and J. P. Weinman. *Am. J. Physiol.* 139: 233, 1943.
24. Kesić, B. and V. Häusler. *A. M. A. Arch. Indust. Hyg. & Occup. Med.* 10: 396, 1954.
25. Myers, V. C., H. H. Beard and B. O. Barnes. *J. Biol. Chem.* 94: 117, 1931.
26. Hartman, R. H., G. Matrone and G. H. Wise. *J. Nutrition* 57: 429, 1955.
27. Matrone, G., R. H. Hartman and A. J. Clawson. *J. Nutrition* 67: 309, 1959.
28. Kay, H. D. *J. Biol. Chem.* 89: 235, 1930.
29. Rosenthal, O., J. C. Fahl and H. M. Vars. *J. Biol. Chem.* 194: 299, 1952.
30. Snyder, F. H. and W. R. Tweedy. *J. Biol. Chem.* 146: 639, 1942.
31. Wachtel, L. W., C. A. Elvehjem and E. B. Hart. *Am. J. Physiol.* 140: 72, 1943.
32. Hurley, L. S., G. J. Everson and J. F. Geiger. *J. Nutrition* 67: 445, 1959.
33. Roche, J. and N.-V. Thoai. *Adv. Enzymol.* 10: 83, 1950.
34. Friedman, M. H. F. *J. Nat. Cancer Inst.* 13: 1035, 1953.



Arch. Path., 73: 400-403

Effect of Hard Water and $MgSO_4$ on Rabbit Atherosclerosis

JOHN E. NEAL, M.D., AND MARYBELLE NEAL, B.S., JACKSONVILLE, FLA.

Schroeder[1] recently reported that a statistical study showed that the states and large cities with the hardest water had a lower mortality rate for coronary heart disease than states and large cities with softer water. They also found from their statistical studies that there were negative correlations (protective effect) with contents of magnesium, calcium, bicarbonate, sulfate, fluoride, and dissolved solids, the specific conductivity of water, and especially with pH. No significant correlations were found between death rate and contents of silica, iron, sodium and potassium, chloride, the ratio of sulfate to bicarbonate, nitrate, manganese, carbonate, color, turbidity, or the number of treatments given water. There was no correlation with diseases other than cardiovascular disease.

Vitale and his associates[2,3] found that magnesium oxide (MgO) given orally caused a reduction in atherosclerosis in rats on a high-fat diet. They also found that a high-fat diet caused magnesium deficiency in rats not given more magnesium than the usual requirement on a regular diet. They also found the magnesium had no effect on blood cholesterol and that the magnesium-fed rats were larger than the others.

Before the present experiments described here, we did several experiments lasting 2 months each, using New Zealand rabbits fed on rabbit pellets and 1% cholesterol. Our results showed only very minimal atherosclerosis of the rabbit aortas. In these rabbit experiments which showed very minimal atherosclerosis, we had used hard sulfur water from our artesian well. To see what results different waters have on rabbit atherosclerosis, we did the experiments described below.

Submitted for publication May 2, 1961.

Materials and Methods

For the first experiment, we used New Zealand rabbits divided into 4 main groups of 4 rabbits each. All 4 groups were given Purina rabbit pellets, 1% crystalline cholesterol, and 5% butter. Group 1 was given distilled water. Group 2 was given untreated hard sulfur water from our artesian well. Group 3 was given hard chlorinated water (Jacksonville City Water); see Table 1. This water is obtained from deep artesian wells and aerated to remove the H_2S. The different analyses on Table 1 are from the different wells that combine to supply the city. Group 4 was given hard nonsulfur water from a rock well. We gave all rabbits Sulfa-Nox (solution of sulfaquinoxaline sodium), one teaspoonful per rabbit mixed in the drinking water on the first day of the experiment to prevent illness.

After 2 months, the rabbits were killed, and the atherosclerosis of the aortas was graded. We used the entire thoracic and abdominal aortas and graded the amount of the atherosclerosis 1+, 2+, 3+, 4+. Representative sections of aortas and coronary arteries were studied microscopically.

Results

The aortas of the rabbits on distilled water had more atherosclerosis than those on hard water. It did not make any difference whether the water was hard sulfur, hard chlorinated, or nonsulfur hard water. The groups on hard water had less atherosclerosis than the group on distilled water. All aortas in all the groups showed some atherosclerosis. There was more in the thoracic aorta and less in the abdominal aorta. Several 4+ aortas of the distilled water group had fat plaques all the way down the aorta. The 1+ aortas in the hard water group had very small minimal plaques. From time to time in the past several years, Dr. Alvan G. Foraker, Pathologist, Baptist Memorial Hospital, Jacksonville, Fla., and Dr. Leila H. Wells and Dr. Carl H. Wells, Pathologists, Wells Medical Laboratory,

TABLE 1.—*City of Jacksonville, Fla., Municipal Water Analyses, July 21, 1960*

	River Oaks	Hendricks	Mc Duff Ave.	Lakeshore	Main St.	Fairfax	Norwood
1. Station	7-6	7-5	7-6	7-6	7-8	7-7	7-8
2. Date (1960)	8:45A	2:30P	11:15A	1:30P	11:15A	2:30P	8:45A
3. Time of sampling	10:15A	3:00P	12:45P	2:45P	1:30P	3:30P	9:45A
4. Pumping rate mgd	5.9	2.2	13.6	3.0	14.2	9.5	5.4
5. Chlorine Feed #/day	44	30	148	35	148	145	72
6. Chlorine rate #/mg	7.5	13.7	10.9	11.7	21.0	13.8	13.3
7. Chlorine rate ppm	.89	1.64	1.3	1.4	1.77	1.74	1.60
8. Chlorine residual	.75	.87	.60	.35	.75	.45	.70
9. Hydrogen Ion (Ph)	7.9	7.77	7.73	7.9	7.7	7.7	7.75
10. Phenolphthalein alkalinity	0	0	0	0	0	0	0
11. Methyl orange alkalinity	115.3	129.3	126.0	129.0	147.3	138.0	141.3
12. Free carbon dioxide (CO_2)	2.46	4.15	4.07	3.75	4.9	4.6	4.0
13. Oxygen (O_2)	5.65	4.05	6.52	5.91	3.62	5.21	4.4
14. Calcium as $CaCO_3$	199	182	188	128	151	170	143
15. Magnesium as $CaCO_3$	145	132	134	92	115	105	91
16. Total hardness	344	314	322	220	266	275	234
17. Chlorides as Cl	23	27	23	27	27	21	20
18. Dissolved Iron as Fe	0	0	0	0	0	0	0
19. Sulfate as SO_4	124	309	253	221	209	225	106
20. Silica as SiO_2	18	19	16	16	21	20	23
21. No. of samples taken for avg.	3	3	3	3	3	3	3

Analysis made by Plants Efficiency Department, City of Jacksonville, Fla. (G. Finklea, C. Henley, G. Roese)
Hydrogen sulfide exists in all of the above waters prior to treatment in the amount of 1½ to 2 ppm.
All results in ppm. unless otherwise stated.

Marshall-Taylor Doctor's Building, Jacksonville, have been kind enough to do microscopic examinations on tissue specimens for us. Microscopic studies of the coronary arteries have shown the same degree of atherosclerosis as found in the aorta.

Although the main purpose of the experiment was to observe the effect on the rabbit aortas, we did some blood cholesterols on several groups at the end of the experiment. Blood cholesterols of 2 rabbits of Group 1 (distilled water group) were 2,425 mg. % and 2,050 mg. %. Blood cholesterols of 2 rabbits in Group 2 (hard sulfur water group) were 2,115 mg. % and 1,440 mg. %. The cholesterols were relatively high in both groups.

There has been some criticism of using rabbits for the study of atherosclerosis. Constantinides, Booth, and Carlson [4] have shown that if rabbits on a high-fat diet for 2 months are then allowed to live for 2 years, they develop lesions similar to those seen in humans. The lesions formed after 2 or 3 months are probably similar to fatty deposits seen in young humans.

A second experiment was done to extend the observations on hard water. In the second experiment, we used New Zealand rabbits divided into 4 groups of 5 rabbits each. All groups were given Purina rabbit pellets, 1% crystalline cholesterol, and 5% butter. All rabbits were given Sulfa-Nox (solution of sulfaquinoxaline sodium), one teaspoon per rabbit in the drinking water on the first day of the experiment to prevent illness. Group 1 was given distilled water and Group II was given hard sulfur water. To evaluate various elements, Group III was given drinking water made by suspending one tablespoon of calcium carbonate in one gallon of distilled water. (This makes a milky suspension in water because calcium carbonate is not very soluble in water). Group IV was given drinking water made by dissolving 7 tablespoons of magnesium sulfate (Epsom salt) in one gallon of distilled water, or 30,000 ppm. This is much more than is usually found in hard water. Group III on the calcium carbonate water drank more of their water than the Group IV rabbits on magnesium sulfate. This tends to balance some of the difference in concentration. This experiment was continued for 3 months; then all rabbits were killed, and their aortas and hearts were studied. These rabbits were slightly less susceptible

to atherosclerosis than the rabbits in the first experiment. This may have been because we obtained these New Zealand rabbits from a different breeder and probably got some hereditary difference. There are variations between individual rabbits, and Gannan and Smith[7] have shown variation between different breeds of rabbits.

Again we found there was more atherosclerosis on the average in the distilled water group (Group I) than in the hard water group (Group II). Group III rabbits (calcium carbonate) showed moderate atherosclerosis (1+ to 2+), while Group IV rabbits (magnesium sulfate) showed no atherosclerosis. The magnesium sulfate group did not have diarrhea, and they were larger and fatter than the rabbits in the other groups at the end of the experiment. At autopsy these rabbits had more subcutaneous fat than the rabbits not on magnesium sulfate.

Although the main purpose of the experiment was the study of the amount of atherosclerosis in the arteries, some studies were run at the end of this experiment on blood lipids.

Blood cholesterols of 2 rabbits in Group I (distilled water) were 885 mg. % and 1,505 mg. % and both had marked turbidity or "milky" serum. Blood cholesterols of 2 rabbits in Group IV (magnesium sulfate) were 965 mg. % and 1,300 mg. %, and both had slight turbidity or relatively "clear" serum. Blood cholesterols on 2 rabbits in Group III (calcium carbonate) were 2,020 mg. % and 2,965 mg. %, and both had marked turbidity or "milky" serum.

All of these blood serums were very "milky" except the 2 magnesium sulfate blood specimens which were relatively "clear." The 2 distilled water serums ("milky") and 2 magnesium sulfate serums ("clear") had similar blood cholesterols, but there was marked difference in the appearance of the serums. To find out if this difference was due to a difference in other fat components, further studies were run on these serums. (See Table 2.)

TABLE 2.—*Comparison of Two Typical Blood Serums*

	A "Milky" Distilled Water Serum, Mg. %	A Relatively "Clear" Magnesium Sulfate Serum, Mg. %
Cholesterol	1,505	1,300
Phospholipids	386	433
Triglycerides	120	508
Total lipids	2,011	2,241

Comment

These experiments showed some protective effect of hard water on rabbit atherosclerosis as compared to distilled water. Large amounts of calcium carbonate dissolved and suspended in distilled water gave no protective effect against rabbit atherosclerosis, while large amounts of magnesium sulfate dissolved in distilled water gave complete protection against rabbit atherosclerosis. The relatively "clear" serum of rabbits drinking distilled water with magnesium sulfate added had essentially the same amount of fat (cholesterol, triglyceride, and phospholipid) as the turbid or "milky" serum of rabbits drinking distilled water. Vitale[2,3] found similar results in the rat by feeding MgO orally as we did in rabbits feeding $MgSO_4$ orally. They did not mention the appearance of the serum or lipemia, but they found in the rat as we did in the rabbit that there was no effect on blood cholesterol, although there was a reduction in atherosclerosis and the magnesium-fed animals were larger.

When the blood serums from the rabbits in this experiment were allowed to stand for one day, the fat particles settled out in the "milky" serum, but the fat in the "clear" serums of the magnesium sulfate rabbits did not settle out. When a slight to moderate amount of magnesium sulfate was added to the "milky" serum in the test tube there was some clearing of the serum, but when a very large excess of magnesium sulfate was added, there was clouding of the serum. Repeated large doses of magnesium sulfate in humans can have toxic effects.[6]

There are several important questions raised by the experiments reviewed and re-

ported. What is the protective substance or substances in hard water? Is this a nonspecific effect of ions on the stability of colloidal fat? The ability of dispersed particles to remain in the colloidal state is greatly affected by the concentration of ions.[7] Colloids, especially hydrophobic colloids such as colloidal fats, can adsorb ions on their surfaces which increase their stability at certain ion concentrations. The charged colloids repel each other. Or is this a specific effect of magnesium on fat metabolism? Further studies will have to be done using $CaCl_2$ and other ionizing compounds to answer these questions.

Parsons[8] recently reported a study in patients with coronary artery disease giving a restricted-fat diet, with 2 ml. of magnesium sulfate and 5,000 units of heparin intramuscularly weekly for up to one year. They found a sharp fall in total lipids, β-lipoproteins and protein-bound cholesterol. They state that heparin causes a marked clearing of lipemia, and administration of magnesium sulfate reduces coagulability and lowers β-lipoproteins while increasing fibrinolytic activity. They suggest that parenteral magnesium sulfate and minimal doses of heparin might be used to treat coronary artery disease. Further study should be done on the effect of these substances separately and combined on blood lipids and atherosclerosis. Electrolytes other than magnesium sulfate should also be studied.

Summary

Several experiments with New Zealand rabbits showed that rabbits on a high-fat, high-cholesterol diet had less atherosclerosis in aortas and coronary arteries if they drank hard water than if they drank distilled water. A group of rabbits on a high-fat, high-cholesterol diet drinking distilled water with calcium carbonate dissolved and suspended in the water had moderate atherosclerosis, while rabbits on a high-fat, high-cholesterol diet drinking distilled water with magnesium sulfate dissolved in the water had no atherosclerosis. The blood serums from the distilled water rabbits and the calcium carbonate rabbits were very turbid or "milky," while the serums from the magnesium sulfate rabbits were relatively "clear," although all groups had essentially the same amount of fat in their serums.

We are grateful to Dr. Leila H. Wells and Dr. Carl H. Wells, Pathologists, Wells Medical Laboratory, and Dr. Alvan G. Foraker and his associates in the Pathology Department, Baptist Memorial Hospital, Jacksonville, Fla., for microscopic studies. We are grateful to Mrs. Sophie Adams, M.T., Miss Nancy Franklin, M.T., Miss Mary Weakley, M.T., Mr. Don Osborne, B.S., and Mr. Jack Mahoney, M.T., for laboratory studies.

John B. Neal, M.D., Route 8, Box 298, 3720 Julington Creek Road, Jacksonville 17, Fla.

REFERENCES

1. Schroeder, H. A.: Relation Between Mortality from Cardiovascular Disease and Treated Water Supplies, J.A.M.A. 172:1902, 1960.

2. Vitale, J. J.; White, P. L.; Nakamura, M.; Hegsted, D. M.; Zamcheck, N., and Hellerstein, E. E.: Interrelationships Between Experimental Hypercholesteremia, Magnesium Requirement, and Experimental Atherosclerosis, J. Exp. Med. 106:757, 1957.

3. Hellerstein, E. E.; Vitale, J. J.; White, P. L.; Hegsted, D. M.; Zamchek, N., and Nakamura, M.: Influence of Dietary Magnesium on Cardiac and Renal Lesions of Young Rats Fed on Atherogenic Diet, J. Exp. Med. 106:767, 1957.

4. Constantinides, P.; Booth, J., and Carlson, G.: Advanced Atherosclerosis of the Human Type in the Rabbit, Circulation 22:677, 1960.

5. Smith, D. H.: Breed Susceptibility in Rabbits to Hypercholesterolemia and Atherosclerosis, Circulation 20:973, 1959.

6. Gonzales, T. A.; Vance, M.; Helpern, M., and Umberger, C. J.: Legal Medicine: Pathology and Toxicology, Ed. 2, New York, Appleton-Century-Crofts, Inc., 1954, p. 770.

7. Daniels, F.: Outlines of Physical Chemistry, New York, John Wiley and Sons, Inc., 1948, p. 539.

8. Parsons, R. S.: Atheroma as the Result of a Disturbance in the Metabolism of Protein Rather Than Fat, Med. Proc. 6:479, 1960.

Turkish Journal of Pediatrics
6: 211-217, 1964

EFFECT OF MAGNESIUM SULFATE ON BLOOD SUGAR LEVELS OF DIABETIC CHILDREN

Şinasi Özsoylu, M.D.*

Although insulin is still the drug of choice in the treatment of diabetes in children, oral hypoglycemic drugs have been widely used in the control of adult diabetes. Rubenstein, et al. have recently reported on the hypoglycemic affect of manganese in a child with an unusual form of diabetes of seven years duration.[1] Since magnesium is known to be necessary in several pathways of carbohydrate metabolism and is closely related chemically to manganese, magnesium sulfate infusion was given to three diabetic children who were on insulin treatment at Hacettepe Children's Hospital. Although Rubenstein et al. used very small amounts (5 mg) of manganese by mouth, we gave large amounts of magnesium sulfate infusions (150 mg/Kg) intravenously. This is equal to 20 mg/Kg of magnesium.

The ages of the diabetic children were from 5 to 14 years. Two of them were admitted for the first time to the hospital for treatment of their initial diabetic acidosis. The third child (5 years old) had been followed in this hospital for more than three years for diabetes. The acidosis was treated according to Dr. Hartmann's method[2] and magnesium sulfate infusions were given after the diabetes was under moderate control, i.e., when total urinary sugar output was around five per cent of total available glucose.

For three consecutive days prior to magnesium sulfate infusion, fasting blood sugar levels were determined at 8 a.m. Then the daily insulin requirement was given in the form of a mixture of crystalline and NPH insulin, after which the patients had breakfast. Blood was drawn for blood sugar determinations again at 8:30, 9:00 and 9:30 a.m. This procedure was followed on the fourth day, with the addition of a 3 per cent solution of magnesium sulfate (150 mg/Kg) given intravenously while the patient breakfasted. The magnesium sulfate

From Ankara University Hacettepe Faculty of Medicine and Hacettepe Children's Hospital.

* Hematologist and Associate in Pediatrics.

was given as rapidly as possible, within a period of 20 to 30 minutes. Blood was obtained again at 8:30, 9:00, and 9:30 a.m. During these four days, the patients' diets and insulin amounts were not changed. Blood sugar determinations were done by the Folin-Wu method[3] and the results are summarized in Table 1.

TABLE 1

BLOOD SUGAR LEVELS OF THREE DIABETIC CHILDREN ON THREE CONTROL DAYS AND AFTER INTRAVENOUS MAGNESIUM SULFATE INFUSIONS

	Fasting Blood Sugar 8:00 AM	Blood Sugar 8:30 AM	Blood Sugar 9:00 AM	Blood Sugar 9:30 AM
I.Y.				
Ist day	155 mg %	160 mg %	122 mg %	170 mg %
2nd day	262 mg %	258 mg %	222 mg %	190 mg %
3rd day	219 mg %	254 mg %	272 mg %	282 mg %
4th day*	226 mg %	169 mg %	127 mg %	111 mg %
M.S.				
Ist day	338 mg %	282 mg %	242 mg %	295 mg %
2nd day	418 mg %	434 mg %	414 mg %	tube broken
3rd day	400 mg %	416 mg %	432 mg %	390 mg %
4th day*	392 mg %	208 mg %	197 mg %	230 mg %
I.B.				
Ist day	174 mg %	225 mg %	316 mg %	—
2nd day	64 mg %	200 mg %	308 mg %	310 mg %
3rd day	406 mg %	420 mg %	—	385 mg %
4th day*	550 mg %	432 mg %	410 mg %	316 mg %

* The day that magnesium sulfate infusion was given.

Magnesium sulfate infusions were given to three patients without diabetes as a control measure. These patients had urinary tract infection, iron deficiency anemia, and ecchynococcus of the lungs, respectively. Family history of diabetes was not obtained for any of the control cases. Blood sugar determinations of the control group were done at the same hours as the diabetic patients and the same procedure was used in administering the magnesium sulfate solution on the fourth day. The results of the blood sugar determinations for this group are given in Table 2.

TABLE 2

BLOOD SUGAR LEVELS OF CONTROL CASES PRIOR TO AND ON THE DAY MAGNESIUM SULFATE WAS INFUSED (IN MILLIGRAMS %)

Patient	Fasting Blood Sugar 8:00 AM	Blood Sugar 8:30 AM	Blood Sugar 9:00 AM	Blood Sugar 9:30 AM
M.Y.				
Ist day	70	92	87	92
2nd day	89	92	92	77
3rd day	83	77	91	85
4th day*	83	97	105	tube broken
H.E.D.				
Ist day	88	110	108	99
2nd day	98	82	95	98
3rd day	82	110	102	110
4th day*	90	99	103	91
T.Y.				
Ist day	112	111	118	108
2nd day	100	105	90	108
3rd day	87	110	120	103
4th day*	95	107	118	133

* The day that magnesium sulfate infusion was given.

Results

As can be seen in the tables, magnesium sulfate infusion did not have any effect on normal blood sugar levels in the control cases. However, blood sugar levels of all three diabetic patients dropped considerably (116 mg %, 195 mg %, and 234 mg %, respectively) within one hour following magnesium sulfate infusion. It seems that the higher the blood sugar level, the greater the effect of magnesium sulfate in decreasing blood sugar.

Discussion

Magnesium is second in abundance only to potassium as an intracellular cation known to be an activator in several pathways of carbohydrate metabolism.

The pathways in which the magnesium ion has some role, Embden-Meyerhof, glucose storage, and pentose shunt, are indicated in Figure 1. The magnesium ion is also required in the citric acid cycle (succinyl CoA $\xrightarrow{Mg^{++}}$ succinate). The magnesium ion is known to be necessary

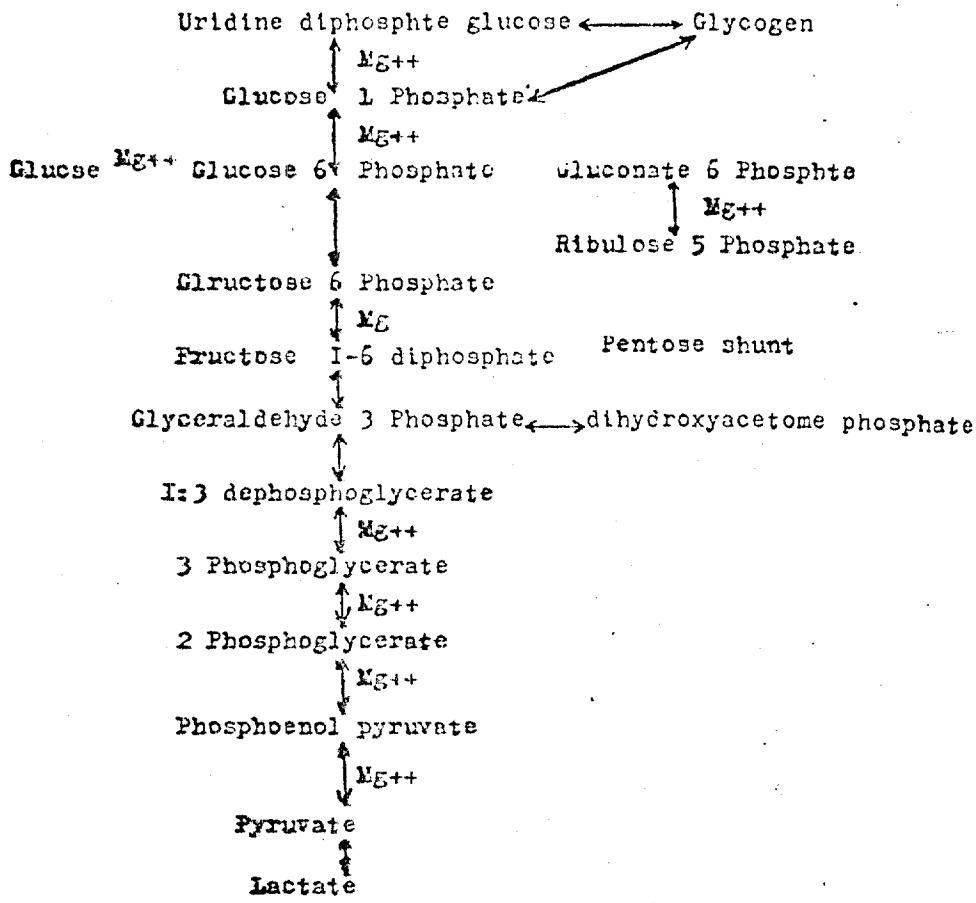


Figure 1. Pathways of glucose metabolism in which magnesium ion is the activator.

for the activation of phosphoralase, phosphatase, enolase, peptidase, decarboxilase, and those enzymes which require ATP. Thus it has not been possible to prove an absolute requirement for this ion, and, on this basis, a definitive physiologic role for magnesium cannot be assigned.[4]

Normal serum magnesium levels have been determined for children and newborns.[5,6] Lower than normal levels have been observed in malnutrition,[6,7] hyper and hypoparathyroidism,[8] hyperaldosteron-

ism,[9] celiac disease,[10] malabsorption syndrome,[11] and following treatment for diabetic acidosis.[12-13]

Magnesium is absorbed from the small intestine and, as for calcium, increased phosphate intake and magnesium soap decrease magnesium absorption. Vitamin D however has no effect on the absorption of magnesium.[4] Fifty per cent of the body's magnesium is stored in the bones, and most of the remainder is intracellular.[14] Thirty-five per cent of the magnesium in plasma is bound to protein, and a small amount is bound to citrate.[4] The largest proportion of magnesium in plasma is in its ionized form. Excretion of magnesium occurs through the kidneys. Lactate decreases magnesium re-absorption through the proximal convoluted tubules, which is one cause of magnesium deficiency during the treatment of diabetic acidosis if lactate is used. Although Diamox[R] (Acetazolamide; 2-acetylamino-1,3,4,-thiadiazole-5-sulfonamide) increases calcium excretion through the kidneys, it decreases magnesium excretion. Parathormone effect on magnesium metabolism is similar to that on calcium metabolism. Magnesium has a pharmacologic effect on the cardio-respiratory system, the central nervous and the myoneural junctions of the muscles.[15] Eosinophilia and proximal tubular changes due to magnesium deficiency is reported in rats.[16,17]

Since manganese proved to be effective in controlling blood sugar levels of at least one diabetic patient, and since the effect of magnesium on carbohydrate metabolism can be partially replaced by manganese, and the mean serum magnesium concentration of patients with controlled diabetes receiving insulin is said to be low,[12] magnesium sulfate infusions were given to those diabetic children with high blood sugar levels. Although the data are not sufficient to support a definitive conclusion, it seems that magnesium sulfate is effective in decreasing high blood sugar levels.

Rubenstein and his colleagues[1] gave magnesium to their manganese-sensitive diabetic patient in order to ascertain whether the patient's response to manganese was specific, without any effect on his blood sugar level. The magnesium was given orally and the dose was not specified. Although Garby and de Verdier[18] did not show the effect of various concentration of magnesium on erythrocyte glucose consumption *in vitro*, it has been shown that glucose uptake by isolated rat diaphragm is increased in the absence of, but more so in the presence of, insulin.[19] Rubenstein et al postulated that manganese might act in one of two ways:

1. It may act peripherally, and may accelerate cellular glucose uptake or utilization either directly or by potentiating insulin action; or it may interfere with the action of glucagon in forming glucose from stored glycogen in the liver.
2. Manganese may act on the pancreas by accelerating the release of stored insulin into the bloodstream, or by inhibiting the release of glucagon.

In explaining the effect of manganese on their patient, they favored inhibition of the release of glucagon.

When blood glucose levels of diabetic children are in reasonably good control, glucagon produces a hyperglycemic effect as in a normal person. However, when the blood sugar is markedly elevated, the effect of glucagon is less predictable and might depress blood sugar levels.[20] For that reason, without further evidence, it seems more reasonable to suppose that the glucose uptake and storage in the cells is increased by magnesium infusion. Unfortunately, magnesium levels could not be measured in our cases. In one of these (I.Y) magnesium sulfate was given orally (0.5 gm) twice a day, b.i.d. with some decrease in his insulin requirement (about 10 units) at the beginning. Since we do not see many diabetic children in Turkey, more data could not be collected on this subject. We thought it worth publishing this preliminary data with the hope that it would interest other investigators.

Summary

The effect of magnesium sulfate on the blood sugar levels of three moderately well-regulated diabetic children is reported. With magnesium sulfate infusion in all these cases initial high blood sugar levels decreased considerably. Magnesium sulfate was not effective at all in three children with normal blood sugar levels.

REFERENCES

1. Rubinstein, A.H., Levin, N.W., and Elliott, G.A.: Manganese induced hypoglycemia, Lancet **2**: 1348, 1962.
2. Hartmann, A.F., Sr., and Klingberg, W.G.: Diabetes Mellitus in Infants and Children: Report of the Twelfth Ross Conference on Pediatric Research, Columbus, Ohio, Ross Laboratories, 1955, p. 41.
3. Harrison, G.A.: Chemical Methods in Clinical Medicine, fourth edition, J. and A. Churchill Ltd, 1957, p. 188.
4. Wacker, W.E.C., and Vallee, B.L.: Magnesium metabolism, New England J. Med. **259**: 431, 1958 **259**: 475, 1958.
5. Anast, C.S.: Serum magnesium levels in the newborn, Pediatrics **33**: 969, 1964.
6. Mays, J.E., and Keele, D.K.: Serum magnesium levels in healthy children and in various disease states, A.M.A.J. Diseases of Children **102**: 623, 1961.
7. Back, E.H., Montgomery, R.D., and Ward, E.E.: Neurological manifestations of magnesium deficiency in infantile gastroenteritis and malnutrition, Archives of Diseases in Childhood **37**: 106, 1962.
8. Homer, L.: Hypoparathyroidism requiring massive amounts of medication with apparent response to magnesium sulphate, J. Clinical Endocrinology **21**: 219, 1961.
9. Mader, I.J., and Iseri, L.T.: Spontaneous hypopotassemia, hypomagnesemia, alkalosis, and tetany due to hypersecretion of corticosteriod-like mineral corticoid, American J. Medicine **19**: 976, 1955.
10. Goldman, A.S., Van Fossan, D.D., and Baird, E.E.: Magnesium deficiency in celiac disease, Pediatrics **29**: 948, 1962.
11. Booth, C.C., Babouris, N., Hanna, S., and MacIntyre, I.: The incidence of hypomagnesemia in intestinal malabsorption, British Medical Journal **2**: 141, 1963.
12. Stutzman, F.L., and Amatuzio, D.S.: Blood serum magnesium in portal cirrhosis and diabetes mellitus, J. Lab and Clin. Med. **41**: 215, 1953.
13. Butler, A.M., Diabetic Coma, New England J. Med. **243**: 648, 1950.
14. Widdowson, E.M., McCance, R.A., and Spray, C.M.: Chemical composition of human body, Clinical Science **10**: 113, 1951.
15. Vallee, B.L., Wacker, W.E.C., and Ulmer, D.D.: The magnesium deficiency tetany syndrome in man, New England J. Med. **262**: 155, 1960.
16. Hungerford, G.F., and Karson, E.F.: The eosinophilia of magnesium deficiency, Blood **16**: 1642, 1960.
17. Barron, G.P., Brown, S.O., and Pearson, P.B.: Histological manifestation of magnesium deficiency in rat and rabbit, Proc. Soc. Exper. Biol. and Med. **70**: 220, 1949.
18. Garby, L., and de Verdier, C.: Glucose metabolism in normal erythrocytes I. Kinetics of the hexokinase reaction in intact cells, Scand. J. Haemat. **1**: 150, 1964.
19. Bhattacharya, G.: Effects of metal ions on the utilisation of glucose and the influence of insulin on it by the isolated rat diaphragm, Biochem. J. **79**: 369, 1961.
20. Etzwiler, D.D.: Variation in glucagon response among juvenile diabetics, Pediatrics **32**: 1002, 1963.

J. Jamaican Assn. Sugar Technol, 27: 48-58, 1966

MAGNESIUM OXIDE IN THE RAW SUGAR HOUSE

by

Jerrold R. Radway

Magnesium oxide is finding increasing acceptance around the world as a partial or complete substitute for lime in sugar cane juice clarification. Magnesium oxide reduces overall costs by increasing capacity of the juice heaters, clarifiers, mud filters, and evaporators. The savings are achieved despite a higher purchased cost for magnesium oxide. In some cases, magnesium oxide also reduces molasses production and improves sugar quality.

Because magnesium oxide is different from lime, it can provide the advantages mentioned earlier. Just what are the differences between magnesium oxide and lime that bear on raw sugar processing? As summarized in Figure I, they are.

1. Lime reacts with sucrose to form a soluble saccharate. Magnesium oxide does not.

2. The solubility of most magnesium salts is higher than the same calcium salts. The saccharate mentioned above is one exception.

3. Magnesium oxide has a higher neutralizing power per unit weight.

4. The apparent reaction rate of magnesium oxide is somewhat slower than that of lime.

Some of the reactions between lime and sucrose are shown in Figure II, together with selected solubility data.

The reaction of calcium oxide and sucrose to form a soluble saccharate is the basis of an old method for separating magnesium oxide from the mineral dolomite (intimate mixture of calcium and magnesium carbonates). The dolomite was calcined, hydrated, and mixed with sucrose to dissolve the calcium as the saccharate. The magnesium oxide was then removed by filtration and the sucrose recovered from the saccharate by carbonation.

The significance of this difference between calcium oxide and magnesium oxide for sugar processing is obvious. Sugar losses should be lower when using magnesium oxide since it doesn't form a saccharate. "When $Ca(OH)_2$ is added to a solution of sugar (sucrose), soluble calcium sucrates are formed until about 2 mols of $Ca(OH)_2$ are added for each mol of sugar present in solution".[1] This would be equivalent to a maximum of 0.43 lbs. of lime hydrate per pound of sucrose. Every pound of calcium oxide can tie up from two to six pounds of sucrose, depending on which saccharate is formed. Khavalkovskii[2] has confirmed the advantage of magnesium oxide by adding calcium and magnesium compounds to a standard molasses and then adding pure sugar and crystallizing. He concluded that calcium was more melassigenic than magnesium.

The presence of ions such as sulfate, phosphate, and oxalate, which compete with the sucrose for the calcium oxide, tend to reduce the sucrose lost to molasses. However, these ions cause scaling problems. Calcium sulfate, calcium phosphate, and calcium oxalate are generally the major constituents in evaporator scales, along with occluded silica and organic matter. Composition of some typical scales is shown in Figure III. Puerto Rican and Florida mills must contend with calcium sulfate scales while Louisiana mills are troubled with calcium phosphate deposits. The Barbados scale was taken after use of a 60% magnesium oxide liming slurry. The predominance of calcium sulfate and oxalate in the scale indicates that insufficient magnesium oxide was used. The Jamaican scale is representative of the very light deposits which are occasionally produced when magnesium oxide is substituted for lime. Such deposits do not measurably decrease operational efficiency. Note that the deposit is free of the usual calcium phosphate and consists largely of organic matter with lesser quantities of silica.

Most magnesium salts are more soluble than calcium salts. As shown in Figure IV, there is a considerable difference in the solubility of the salts of calcium and magnesium. The obvious implication is that during evaporation, the calcium compounds will precipitate from the sugar solution as scale at lower concentrations than will the comparable magnesium compounds.

Because of its lower molecular weight, magnesium oxide has a much greater neutralizing power per pound than either quick-lime or hydrated lime. Figure V depicts for various lime-magnesium oxide combinations, the weights of each which will be required to provide neutralization equivalent to 100% lime. It is evident that the total weight of the two materials is always less than that for lime alone. The higher neutralizing power is important from the economic standpoint since it tends to offset the higher cost of the magnesium oxide. More important, it means lower ash concentrations in the juice because less material would be added to achieve a given pH. In view of the rule of thumb that sucrose loss to molasses is five times the inorganic non-sugars, the higher neutralizing power should also favor improved yields.

Magnesium oxide reacts more slowly than lime. The curves in Figure VI are for simulated sugar juice, acetic acid buffered with ammonium acetate. Note that when Magox is added on an equivalent hydroxyl basis it reaches approximately the same terminal pH, but the rate of rise is somewhat slower.

The slower reaction of the magnesium oxide must be considered in controlling pH. Residence time ahead of the pH control in the liming circuit, Figure VII, should be sufficient to insure that the magnesium oxide will have reacted by the time the juice reaches the pH electrode. Otherwise, the measured pH will be low, the control will call for increased lime slurry, and the excess base will react downstream causing high pH. According to Honig,[3] the solubilities of lime salts increase with pH and losses to molasses could also be expected to increase in accordance with the "rule of thumb" on inorganic non-sugars. Thus, failure to consider the difference in reaction rate can negate the potential advantage of lower molasses losses with magnesium oxide. Fortunately, such simple changes as lowering the set point on the controller or measuring pH at the discharge of the juice heater can insure that such advantages are realized.

Lime generally costs less than magnesium oxide, so economics dictate that magnesium oxide be applied in a judicious manner. The optimum point is that magnesium oxide substitution for lime which will restrict the calcium concentration to slightly less than the limit of solubility under the conditions in the final evaporator effect. The use of more magnesium oxide would raise the chemical cost without additional benefit while the use of more lime would result in evaporator scaling. Excessive amounts of lime would further result in sucrate formation and increased loss

of sucrose to molasses.

The optimum proportion of calcium oxide to magnesium oxide in the liming slurry will vary with the juice and depend on the juice pH and the concentrations of calcium oxide, sulfate, phosphate, or oxalate already present in that juice. The relative concentrations of the various ions and the concentration ratio through the evaporator will govern whether or not one or more of the solubility limits are exceeded in the final evaporator effect.

It is apparent from the preceeding discussion on balancing the quantities of calcium oxide and magnesium oxide used in liming that two kinds of data are necessary for accurate adjustments; solubilities of the calcium salts of the scale forming ions under factory conditions and the anticipated concentrations after evaporation. The solubilities are difficult to assess because of the complexity and the variability of the syrup composition. Similarly, present analytical methods do not afford rapid determination of concentrations of the pertinent ions. Hopefully, new techniques such as the "specific ion" electrode or atomic absorption will soon resolve these analytical problems. While we cannot accurately predict the balance between calcium oxide and magnesium oxide because of the limitations of our present techniques, we can use theory to guide our application.

We have stated the advantages of using magnesium oxide, emphasizing both the differences between lime and magnesium oxide, and the part the differences play in realizing or not realizing these advantages. With those differences firmly in mind, some amplification of the advantages seems appropriate. Mention was made of increased capacity of the juice heaters. Since less calcium is present, scale and fouling from inorganic salt deposits are reduced and heat transfer efficiency is maintained.

Improvements in clarifier, mud filter, and evaporator capacities have either been reported or suggested in papers presented by Hamilton and Payne,[4] at the ISSCT meeting last year, and by Hoffman, Smith and Twigg[5] at the ASSCT meeting in June. Hamilton and Payne in their paper and Hugot[6] in his book have stated that use of magnesia may have adversely affected juice clarity. Dr. Payne has since indicated in a personal communication that clarity is not a good measure of the quantity of non-sugars removed in defecation. If clarity has been affected, the use of magnesium oxide certainly has not impaired sugar quality. At least two mills in Hawaii using 100% magnesium oxide are receiving a premium for the high quality of their product.

An adverse effect of magnesium oxide on juice clarity has been seen in our own laboratory work with juices in Louisiana, Puerto Rico, Antigua and Jamaica. However, that work has shown that juice clarity is not necessarily a good measure of the quantity of non-sugars removed during defecation. We ran a series of tests with various proportions of lime and Magox, adding the mixtures on a constant hydroxyl basis. The data in Figure VIII are for Louisiana juices, but the others showed similar trends. In these tests the muds were collected, dried, weighed, and ignited. It was possible to obtain better juice clarity with lime-magnesium oxide mixtures than with lime alone, but clarity was shown to be a poor measure of the removal of total non-sugars. Using the quantity of mud collected as a gauge of defecation efficiency, the appropriate mixture of lime and magnesium oxide was just as good or better than 100% lime, juice clarity to the contrary. In this series of tests, quantities of inorganic matter precipitated were identical for the lime only and the 40% Magox-lime mixture. Since less total base was added with the lime-Magox mixture, the total hardness of the juice should be, and was, less than with lime alone. The lower hardness values would suggest smaller losses to molasses using the industry "rule of thumb". The hardness values are generally consistent with the quantities of inorganic matter removed. Increased clarifier capacity is indicated by the faster settling rate and smaller mud volume. Higher mud densities should provide faster filtration on the rotary vacuum equipment.

The foregoing results suggest several techniques which might be useful in optimizing lime-magnesium oxide proportions for a given juice:

(a) Determine calcium hardness on the clarified juice as a function of lime-magnesium oxide proportion, selecting that mixture which yields the lowest residual calcium oxide concentration.

(b) Determine the quantity of non-sugars removed during defecation as a function of lime-magnesium oxide proportions selecting that formulation which yields the maximum quantity of mud per unit volume of juice.

The clarification studies under discussion were conducted on a bench scale, but the validity of the conclusions is supported by factory experience. The best testament to that statement is the fact the Emil Hugot, who recommended against use of lime containing as little as 2% magnesium oxide, is now using magnesium oxide in his four factories in Reunion. He has also advised in a personal communication that a short chapter on magnesium oxide will be included in the forthcoming second edition of his book.

Many authors, including Hamilton & Payne , Wotherspoon , and Hoffman-Smith & Twigg , have thoroughly documented the significant value of magnesium oxide in controlling evaporator scaling. Factories all over the world have been able to eliminate formation of hard scale in the evaporators by partial substitution of magnesium oxide for lime in the clarification step. Benefits include maintenance of constant evaporation rate and syrup Brix. The uniform evaporation rate permits maintenance of a constant imbibition water flow, thereby eliminating the normal sucrose loss to bagasse when the flow is decreased because of evaporator scaling. Grinding rates can be maintained at higher levels because of the uniform evaporation rate and elimination of excessive shut-downs for cleaning. Costs for cleaning chemicals are drastically reduced or eliminated and equipment life is extended because damage from mechanical cleaning or corrosion from acid cleaning is similarly reduced or eliminated. Several factories in Reunion reported using 42% to 50% magnesium oxide and grinding the entire season, approximately five months, without scaling or evaporator cleaning. Impressive but not quite as dramatic results have been reported in Jamaica, Mauritius, Puerto Rico, Hawaii, Antigua, Mexico, Louisiana, Florida and Peru. Many of the Hawaiian and Puerto Rican mills have been reaping such benefits for from five to eight years.

The many benefits associated with elimination of evaporator scaling are derived from two of the differences between lime and magnesium oxide discussed earlier; the higher neutralizing power and the greater solubility of the magnesium compounds. The value of the higher solubility of the magnesium oxide compounds was dramatically demonstrated in factory studies conducted by the Mauritius Sugar Industry Research Institute,[8] Figure IX. As the proportion of magnesium oxide in the liming slurry was decreased, the calcium oxide concentration in the clarified juices increased as did the scaling problems. Note that the scaling decreased as the magnesium oxide concentration in the clarified juice increased. Magnesium oxide, though normally found in the juice, is rarely found in the scale.

Use of magnesium oxide in juice clarification has apparently had no effect on vacuum pan or crystallizer operation. There is, however, some conflict in regard to whether or not magnesium oxide causes increased sugar losses to molasses. The only negative report has been a statistical study presented by Mr. Wotherspoon at this meeting last year. In our own experience around the world, magnesium oxide has either yielded a reduction in sucrose losses, or has shown no difference from the lime only controls.

M. Hugot, in a personal communication, has made the following statement in regard to the effects of magnesium oxide on molasses purity. "We did not notice any noteworthy improvement in clarification and in molasses purity, but there might be some". This statement effectively summarizes the results of Basic's statistical study of factory data for the Hawaiian sugar industry for the five year period, 1960 to 1964, Figure X. Factories using lime only had consistently higher percentages of sucrose in molasses. Our statistician indicates that we cannot with confidence conclude from this factory data that magnesium oxide *reduces* molasses losses. However, it is obvious that magnesium oxide does *not* cause an increase in such losses.

The divergence of the conclusions to be drawn from statistical analysis of factory results covering experience over a period of five years and those drawn from the well designed statistical study conducted by Mr. Wotherspoon at Monymusk, led to a review of both efforts. After considerable correspondence and study, I've concluded that the divergence in results can be attributed to the fact that neither Mr. Wotherspoon nor Basic's technical staff considered the effect of the order of addition of the magnesium oxide and calcium oxide on the chemical reactions taking place.

In the tests conducted at Monymusk, the magnesium oxide slurry was added separately and four minutes ahead of the lime. This compares with the standard practice throughout the world of preparing and adding a lime-magnesium oxide pre-mix slurry. As was shown in Figure IV, magnesium phosphate and oxalate are *more* soluble than their analogous calcium compounds. However, that solubility is not so great that prior and separate addition of the magnesium oxide slurry couldn't cause their precipitation from the raw juice. Once precipitated, magnesium phosphate and oxalate, being poorly soluble, would be very unlikely to exchange with calcium compounds added subsequently. Thus the scale forming phosphate and oxalate anions would be partially removed by the magnesium, leaving the calcium ions to form soluble saccharates which are subsequently lost to the molasses.

The curves in Figure XI indicate that magnesium oxide will react with the phosphates in cold raw juice. Calcium oxide reportedly gives only limited reaction with the phosphates before the juice is heated. Honig[9] has attributed the drop in juice pH on heating and clarification to the reaction between lime and phosphate. Typically, allowance is made for the pH drop on heating by employing a set point on the controller somewhat above the pH desired for the clarified juice. An equivalent hydroxyl concentration from magnesium oxide will cause the pH to rise more slowly to a lower plateau, but result in approximately the same clarified juice pH upon heating and clarification. The slower rise and the lower plateau indicate reaction between the magnesium oxide and juice phosphates. Note the similarity between the pH changes occurring with lime hot, and those with magnesium oxide either hot or cold.

Incidentally, the dissimilar paths to the same terminal pH are typical and explain the usual recommendation with lime-magnesium oxide mixtures to lower the set point of the controller 0.3 to 0.5 pH units on the cold juice to avoid overliming.

The premature removal of phosphate by the magnesium oxide leaving calcium ions to form soluble saccharates is probably the major cause of the increase in molasses losses reported by Mr. Wotherspoon. However, overliming is undoubtedly a contributing factor. According to data furnished in personal communications with Mr. Wotherspoon, the estimated quantity of base used during the lime-magnesium oxide runs averaged one third more than during lime only runs. Calcium oxide and magnesium oxide analyses on the sugar and molasses when calculated back to a clarified juice basis, tend to confirm the overliming. Neglecting loss of calcium oxide as scale and assuming 10% sucrose on juice the average hardness for the lime only and lime-magnesium oxide runs would be 515 and 555 p.p.m. respectively. The higher total of calcium oxide and magnesium oxide for the Magox-lime is abnormal. As shown earlier, total hardness in clarified juice is less for lime-magnesium oxide mixtures than for lime alone. Thus, the higher hardness data tend to confirm the overliming indicated by the quantities of lime and magnesium oxide consumed. As mentioned earlier, overliming can cause high calcium salts, high saccharate concentrations, and loss of sugar to molasses.

The fact that the results experienced at Monymusk were divergent from field experience may in the long run prove useful. It has suggested a possible avenue for increasing the efficiency of magnesium oxide in the raw sugar house, i.e. explore the order of adding the magnesium and calcium oxide during liming. Factories faced with high sulfate scale would logically see no effect from modifying the present simultaneous addition procedure. However, those faced with phosphate and oxalate scales should derive benefit from adding the lime prior to magnesium oxide. This eliminates the competition between calcium and magnesium for the phosphate and oxalate ions. The probabilities of these scale forming ions being removed in clarification as the less soluble calcium salt are thereby enhanced. In simultaneous addition, magnesium can precipitate some of these ions, leaving more calcium free to react with sucrose.

In summary, magnesium oxide can offer significant advantages in the clarification circuit of the raw sugar house. These advantages can be realized most consistently and efficiently if one recognizes and adjusts for the differences between lime and magnesium oxide. These differences are:

1. Lime reacts with sucrose to form a soluble saccharate. Magnesium oxide does not.

2. The solubility of most magnesium salts are higher than the same calcium salts. The saccharate mentioned above is one exception.

3. Magnesium oxide has a higher neutralizing power per unit weight.

4. The apparent reaction rate of magnesium oxide is somewhat slower than that of lime.

References

(1) Schollis, Alvin "Economic Considerations in the Recovery of Magnesia from Dolomite" U.S. Bureau of Mines IC 7247 1943, p.12.

(2) Khvalkovskii, T.P. Sokhar. Prom. 1964, *38* 580-583

P. I.S.J. Feb. 1935 p.63

Hamilton, R. K. and Payne, J. H. "Juice Clarification With Magnesia" 12th Congress International Society of Sugar Cane Technologists, San Juan, Puerto Rico, March 1965.

Hoffman, M. S., Smith, M. F., and Twiss, D. J. "The Use of Magnesium Oxide to Prevent Evaporator Scaling in Sugar Factories" American Society of Sugar Cane Technologists June 1966.

) Hugot, E. "Handbook of Cane Sugar Engineering" Elsevier Amsterdam 1960 p.267.

) Wotherspoon, J. R. "Magnesium Oxide in Juice Clarification" J.A.S.T. 1965.

) Vignes, C. and Randabel, J. "The Use of Magox as a Clarifying Agent". Mauritius Sugar Industry Research Institute Annual Report 1964 p.105.

) Honig and Khainovsky I.S.J. 1928 p.264 and Honig I.S.S.C.T. Vol II 1956 p.551.

FIGURE I

ERENCES BETWEEN MAGNESIUM OXIDE AND LIME

	$Ca(OH)_2$	MgO
soluble saccharate?	Yes	No
ility of phosphate, sulfate d citrate salts	Insoluble	Slightly to very
Power per unit weight	1	1.85
tralization rate	Fast	Slower

FIGURE II

REACTIONS BETWEEN LIME AND SUCROSE

$H_{22}O_{11} + Ca(OH)_2 \quad C_{12}H_{22}O_{11} \cdot CaO + H_2O$

$H_{22}O_{11} + 2Ca(OH)_2 \quad C_{12}H_{22}O_{11} \cdot 2CaO + 2H_2O$

$H_{22}O_{11} + 3Ca(OH)_2 \quad C_{12}H_{22}O_{11} \cdot 3CaO + 3H_2O$

OLUBILITY OF $Ca(OH)_2$ IN SUGAR SOLUTIONS

H_2 Dissolved ppm	Sugar in Solution %
1,170	0.00
1,880	.62
7,300	4.82
13,550	7.50
23,100	9.87
32,100	11.90
45,700	15.10
53,800	17.42
[illegible]	19.86

FIGURE III

TYPICAL SUGAR EVAPORATOR SCALE ANALYSIS AND PROJECTED COMPOSITIONS

	Puerto Rico	Florida	Louisiana	Barbados[a]	Jamaica[a]
L. O. I.	36.0	27.0	14.2	38.3	61.6
P_2O_5	1.2	1.1	27.2	.3	2.9
CaO	18.3	25.3	21.8	15.9	5.3
MgO	0.0	2.0	1.3	2	1.7
SO_3	18.2	36.1	8	5.5	2.90
CO_2	0.9	0.		34.4	4
SiO_2	23.0	8.3	9.3	33.42	21.40
R_2O_3	1.9			1.04	

PROJECTED COMPOSITION

$CaCO_3$	2.0				
Organic	36.0	27.0	14.2		61.6
$CaSO_4 \cdot 2H_2O$	31.0	61.4	1.5	11.9	4.9
$CaSiO_3$	6.0				
SiO_2	19.9	8.3	9.3	33.4	21.4
Calcium Phosphate	2.6		50.3[b]		6.3
R_2O_3	1.9				
Calcium Oxalate				32.10	

(a) After Magox-lime mistures (4th effect). The Jamaican sample is representative of the very light deposits which are sometimes obtained with MgO. Such deposits do not measurably decrease operational efficiency.

(b) Based on X-ray crystallography.

FIGURE IV

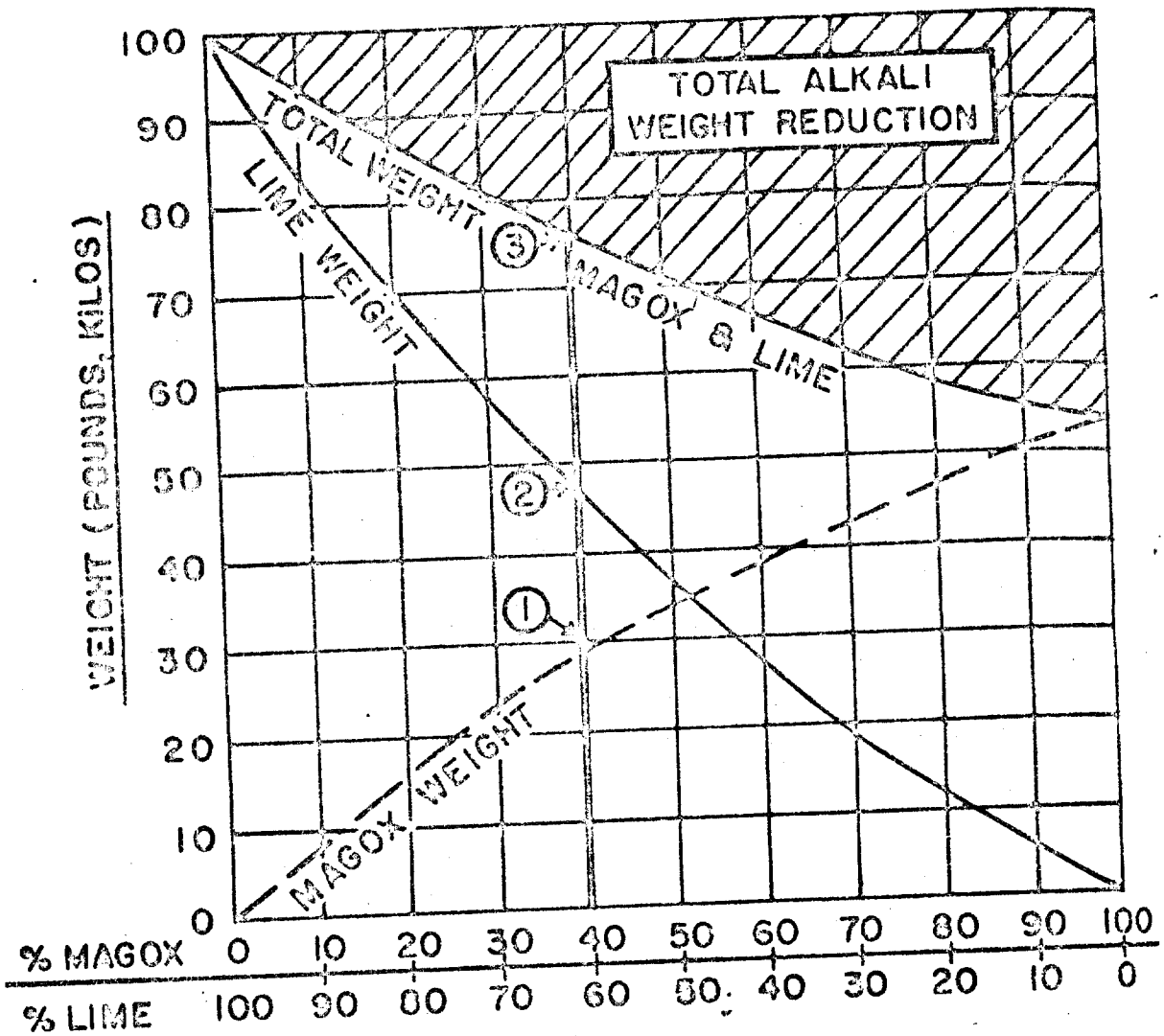
RELATIVE SOLUBILITIES OF CALCIUM AND MAGNESIUM SALTS

gms/100 ml

	Calcium		Magnesium	
Salt	Cold	Hot	Cold	Hot
Sulphate	.223	.257	72.4	178.
Phosphate	.0025	-	.02	-
Oxalate	i	i	.07	.08

FIGURE V

NEUTRALIZING POWER, MgO vs LIME HYDRATE



MAGOX / LIME % COMPOSITION

BASED ON 100 UNITS WEIGHT OF LIME ORIGINALLY USED

FIGURE VI

RATE OF NEUTRALIZATION OF BUFFERED ACETIC ACID WITH HYDRATED LIME AND MAGOX

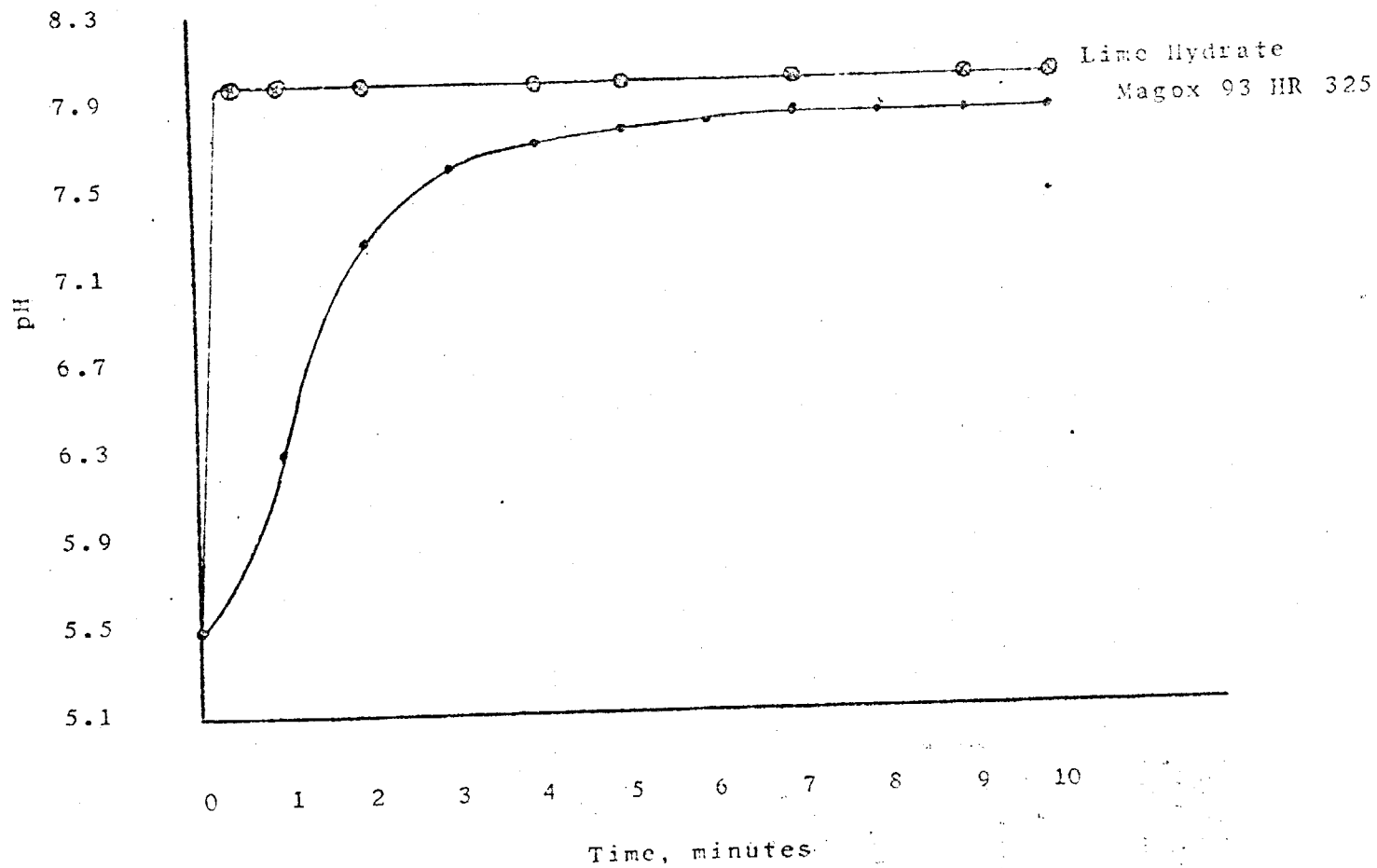


FIGURE VII

TYPICAL LIMING CIRCUIT

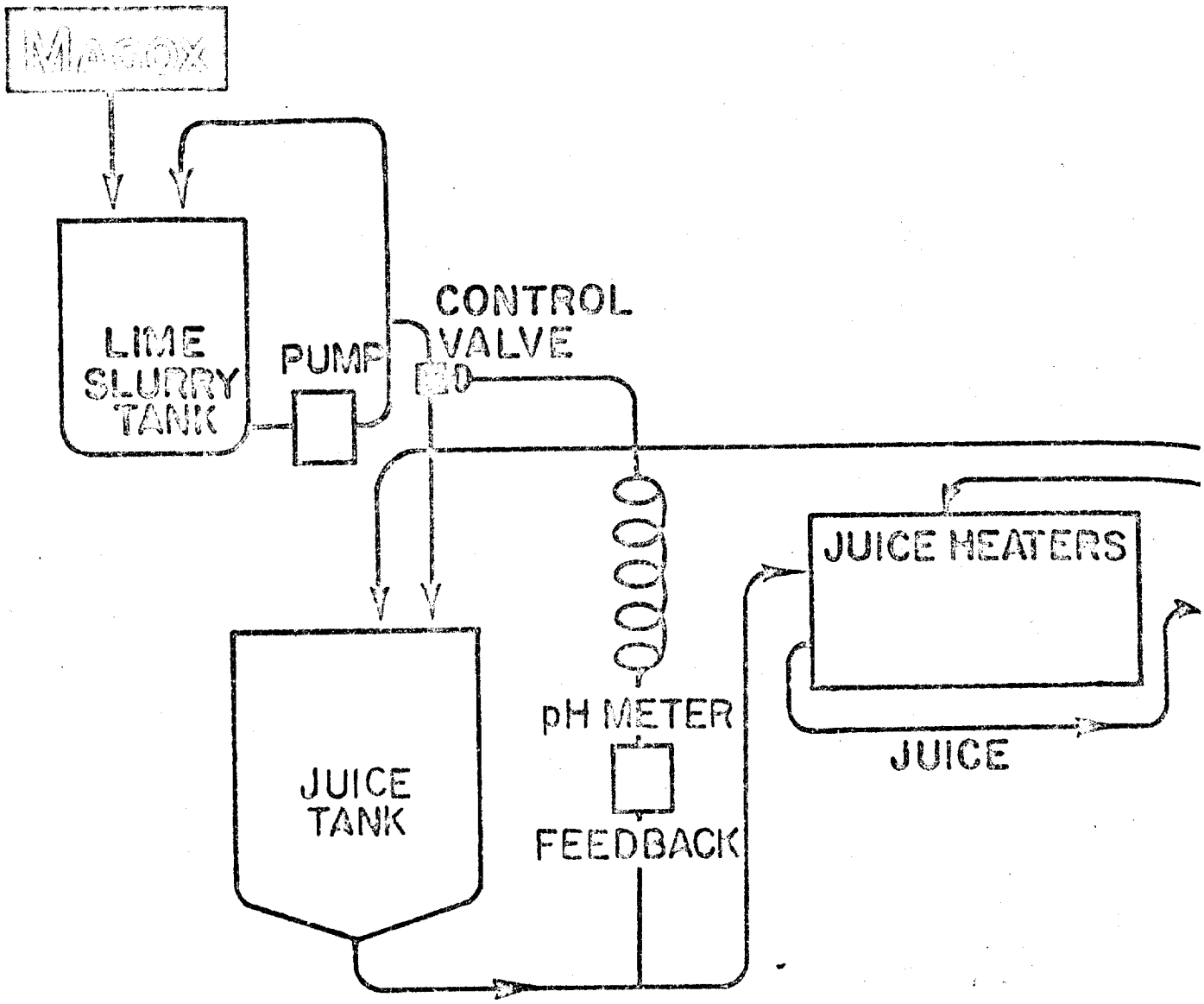


FIGURE VIII

EFFECT OF LIME AND MAGOX-LIME MIXTURES
ON THE CLARIFICATION OF RAW SUGAR JUICE

	Percent Magox in Magox-Lime Mistures				
	100	40	30	20	0
Lime used, gm/500 ml	0	.202	.252	.308	.450
Magox used, gm/500 ml	.242	.134	.106	.077	.0
TOTAL	.242	.366	.358	.385	.450
Juice Clarity % Transmission	0	0.5	6.5	6.5	4.5
Total dry mud, gms/500 ml juice	1.74	2.18	1.92	1.76	2.18
Ignited mud wgt. gms/500 ml juice	1.18	1.50	0.92	0.42	1.50
Mud Composition					
Inorganic, % of juice	0.24	0.29	0.18	0.08	0.30
Organic, % of juice	0.11	0.14	0.20	0.27	0.14
Total Hardness, ppm in clarified Juice	425	350	455	460	460
Mud Volume, cc					
After 15 min.	20	34	35	39	57
After 30 min.	18	34	33	37	52
Mud Density, gm/cc	0.096	0.064	0.058	0.048	0.042

REDUCTION OF SCALE POTENTIAL WITH MAGOX

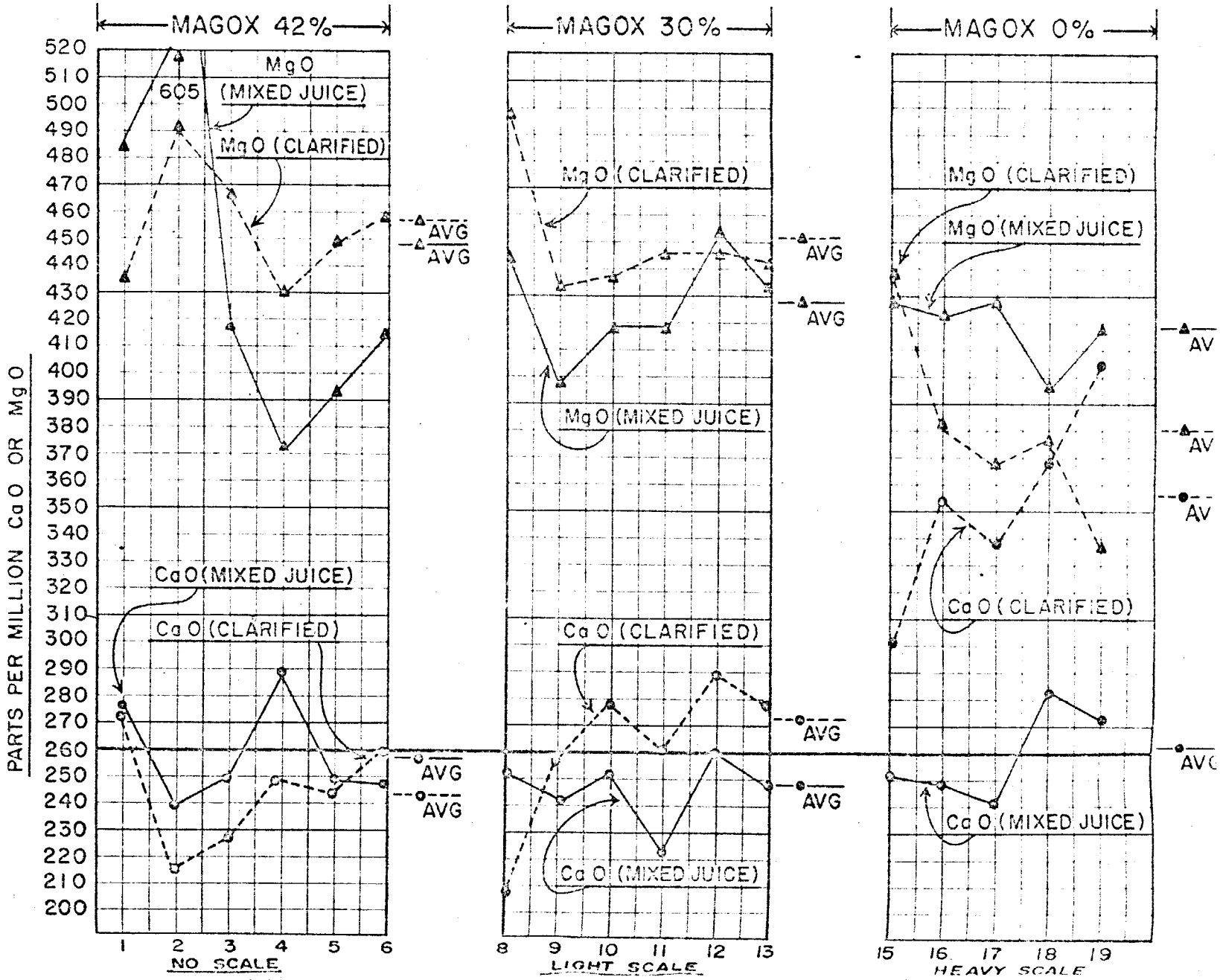


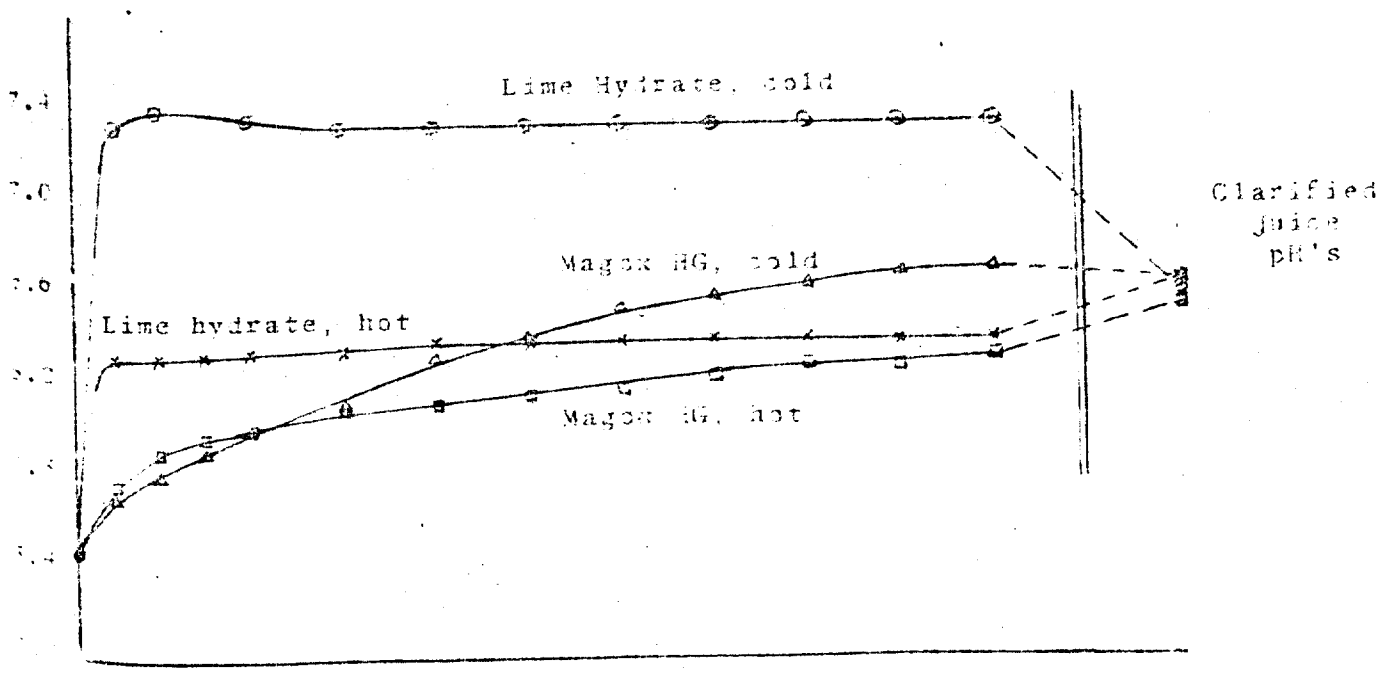
FIGURE X

COMPARISON OF LIME AND LIME-MgO, HAWAIIAN FACTORY DATA

	1960	1961	1962	1963	1964	5 yr. Avge.	# of Mills
Sucrose, % of Molasses							
Lime - MgO	31.84	31.84	31.64	32.04	32.19	31.91	8-10
Lime only	32.18	32.23	32.47	33.00	33.73	32.72	17-19

FIGURE XI

NEUTRALIZATION OF LOUISIANA RAW SUGAR JUICE WITH HYDRATED LIME AND MAGOX



DISCUSSION

MAGNESIUM OXIDE IN THE RAW SUGAR HOUSE

(Mr. P. D. Smith in the Chair)

THE CHAIRMAN thanked Mr. Radway for presenting his interesting paper. He said thanks were due to Mr. J. Wotherspoon who had carried out an evaluation of Magox material during 1965 crop at Monymusk, the results of which had stimulated Mr. Radway to have a further look at the use of magnesium oxide in the Industry. It was regretted that Mr. Wotherspoon was unable to attend the Session.

MR. M. B. FLORO commented on the suggestion that the juice need not be of maximum clarity and wondered whether Mr. Radway had done any analyses of the materials which contributed to the turbidity.

MR. J. E. RADWAY said that the samples had not been analysed and stated that Dr. Payne had found that clarity did not indicate the benefits derived from the use of Magox.

MR. M. B. FLORO stated that it was his experience that with a certain amount of turbidity the fouling of evaporator tubes was reduced and he wondered whether more molasses would be formed if the turbidity caused changes in the composition of the scale.

MR. J. E. RADWAY stated that he had no information on this.

MR. M. B. FLORO suggested that the techniques would be different when dealing with stale cane as opposed to fresh cane.

MR. J. E. RADWAY said that with stale cane, because of the higher concentrations of oxalic acid there would be potentially greater scale formation and one would need a higher proportion of calcium and a lower proportion of magnesium.

DR. M. SMITH said that for stale cane more magnesium oxide/lime mixture would be required to compensate for the lower pH of the juices.

MR. M. B. FLORO enquired what would be the effect of the additional quantities used on the reducing sugars.

DR. M. SMITH said he had no information on this.

THE CHAIRMAN observed that with the great variation in the nature of juices it appeared that adjustments would have to be continually made to accommodate the variations.

MR. M. B. FLORO asked whether there would be subsequent scaling problem in the distillery if there was not a scaling problem in the factory.

MR. J. E. RADWAY said it was still a matter of solubility of the compounds.

DR. M. SMITH said that no increased problems had been encountered in distilleries because of the use of magnesium oxide.

MR. G. MENEZES referred to the fact that Magnesium Oxide increased the turbidity of the juice. Since in stale cane there was an increased quantity of gums and organic material, one would need to get these out of the process as quickly as possible and the use of calcium oxide would be of increased importance in dealing with stale cane. The chemistry of liming was to neutralise the weak acids present in juice and to remove the colloidal impurities in the juice. And since with the use of magnesium oxide the turbidity of the juice was increased then more colloidal impurities would be carried through the process and this could only have an adverse effect on the quality of sugar. This was particularly important at Monymusk where a certain amount of refining of sugar took place.

MR. J. E. RADWAY stated that clarity of the juice did not indicate the removal of organic materials. Better removal of organic materials could be obtained by using magox and lime than lime alone, but this would be done at the expense of evaporator efficiency.

MR. G. MENEZES referred to the trials done at Monymusk in 1964 where the difference in pH of the juices from the various trays in the clarifier suggested overliming. In tests throughout 1965 no differences in pH were observed. This together with direct analyses made them feel that excess base was not used.

MR. J. E. RADWAY mentioned that twice as much base was used with the magnesium oxide/lime mixture than when using lime alone. The difference he thought might have been due to the separate addition of lime and magnesium oxide at Monymusk whereas elsewhere one addition was employed.

MR. G. MENEZES referred to Figure IX and asked whether Mr. Radway would care to say why when no magox was used the level of magnesium oxide in the juice was just as high as in the other two levels.

MR. J. E. RADWAY replied that magnesium oxide was always present in cane juice.

DR. M. SMITH said that the work was done on mixed juice before it went to the liming station.

MR. E. J. MOL pointed out that he thought the term "colloidal solution" should be used instead of colloids. He explained that the normal pH range of 6.0 to 8.0 when using lime was not experienced when using Magox and thought that this might affect the removal of compounds in colloidal solution.

THE CHAIRMAN thanked Mr. Radway for presenting his interesting paper which stimulated much thought and discussion and he thanked the members for their participation.

Forensic Sci. Soc. J. 2 (1) 54 (1962)

A Case of Poisoning by Epsom Salt

P. O. REES

Home Office Forensic Science Laboratory, Nottingham, England

Abnormally high concentrations of magnesium were found in the post mortem blood of a female subsequent to an attempt to produce abortion by the injection of Epsom Salt into the uterus. The literature is reviewed.

Magnesium is the second most plentiful cation in cellular fluids and is an important activator of many enzyme systems in the body. The blood or plasma magnesium levels are maintained within quite narrow limits. Greenberg *et al.* (1933) reported the values: plasma 2.74 ± 0.3; whole blood 4.55 ± 0.38; and red cells 6.61 ± 0.53. Orange and Rhein (1951) reported normal values as: serum 1.6 to 2.5; whole blood 3.7 to 4.7; and red cells 5.9 to 6.8. Thus far there is little information available concerning the mechanisms responsible for maintaining the remarkable constancy of these levels. Deficits in the magnesium ion level of blood and tissue fluids are accompanied by a variety of structural and functional disturbances (Whang and Welt, 1963; Welt and Tosteson, 1964). An excess of magnesium in blood and tissue fluids, i.e., hypermagnesaemia causes depression of the central nervous system and of the peripheral neuromuscular transmission junctions. Less is known about magnesium in clinical conditions than, say, potassium. This is partly due to technical difficulties hitherto encountered in the rapid, yet accurate analysis of plasma and tissue magnesium. Owing to recent advances in techniques for magnesium analysis, e.g., atomic absorption spectrophotometry (Jones and Thomas, 1965), it is likely that in the next few years, more light will be shed on a variety of clinical disorders which may be associated with abnormalities in magnesium metabolism.

When magnesium compounds are taken by mouth as food or as medicine about 30 to 40 per cent. is absorbed, practically regardless of the quantity taken. The absorption occurs chiefly from the small intestine, and excretion is almost exclusively through the kidneys. The sulphate ion acts independently as an osmotic cathartic. Systemic actions of magnesium do not ordinarily occur when magnesium salts are taken by mouth because of the relatively slow absorption and the rapid excretion of the magnesium ion. However, toxic effects may result from very large doses of the sulphate, particularly if kidney injury or disease interferes with elimination (Hirschfelder, 1934). With kidney excision, and sometimes with mercury nephrosis, the plasma can attain the coma level (approximately 17mg per cent.) and it is possible that some "nephritic" comas have been magnesium comas. If saline catharsis is indicated in nephritic cases, sodium sulphate and not magnesium sulphate should be administered. Fatalities have occurred from the administration of Epsom Salt enemas (Fawcett and Gens, 1943), and from hypodermic and intravenous injections of the salt. The chief symptoms are analgesia, anaesthesia, paralysis of the respiration and paralysis of the heart. Failure of natural respiration can occur at serum levels of 18 to 31mg per cent. of magnesium (Moore and Wingo, 1941; Smith *et al.*, 1942). With abrupt injections the heart may be arrested instantly. If the effect of an injection is not fatal, recovery can occur fairly quickly due to the rapid excretion of magnesium. The first and most constant result of an intravenous injection of magnesium sulphate is a fall of blood pressure which continues progressively till death. The blood vessels show marked dilitation. According to Moore and Wingo, analgesia occurs

when the serum magnesium reaches 15mg per cent.; surgical anaesthesia with 23mg per cent.; and respiratory arrest with 33mg per cent. (Moore and Wingo, 1942; Smith *et al.*, 1939, 1942). In the treatment of magnesium poisoning, failure of respiration should be combated by artificial respiration and the recommendations given in the literature, e.g., (Sollman, 1950) are that either 1 to 2mg of physostigmine should be administered subcutaneously or that a 0.02 per cent. solution of calcium chloride, up to a volume of 600ml should be intravenously administered in conjunction with the artificial respiration.

As the concentration of the magnesium ion in whole blood begins to exceed 5mg per cent., deep tendon reflexes decrease and may be absent at levels approaching 12mg per cent. An animal anaesthetized by the intravenous injection of a soluble magnesium salt will regain consciousness and behave normally a few seconds after the intravenous injection of a sufficient quantity of a soluble calcium salt. As yet there is no acceptable explanation of magnesium narcosis or of the antidotal action of calcium. Some investigations conducted by Greville and Lehmann (1943), indicated that magnesium ions suppress the calcium ion activation of the adenosine triphosphate of myosin.

Reports of magnesium poisoning and quantitative details pertaining to magnesium poisoning are rare and the author considered that the particular case he investigated might well be of interest. The case relates to the death of a female where the evidence indicated the entry of Epsom Salt into the blood stream via the uterus. The female, aged 30, and approximately 3 months pregnant, died in circumstances indicating an attempted abortion. A bowl, an enema syringe and a jug lay in the room where the female was found. The bowl contained approximately 12 fluid ounces of a 7.9 per cent. solution of Epsom Salt in water, and traces of Epsom Salt solution were present in the enema syringe and in the jug. The post mortem examination revealed no air-embolism or other obvious cause of death.

The magnesium content of the concentrated Epsom Salt solution in the bowl was determined gravimetrically by precipitating the magnesium as oxinate. This method is inappropriate for magnesium in body fluids, and for these the sensitive Titan Yellow colorimetric method was used. The procedure is an adaptation (Orange and Rhein, 1951) of the method of Garner (1946) and Heagy (1948), and depends on the formation of a red magnesium hydroxide-Titan Yellow lake when a protein-free filtrate is treated with Titan Yellow and sodium hydroxide.

Materials and Method

Titan Yellow	*7.5mg per 100ml H_2O. This should be kept in a cool dark place.*
Sodium Hydroxide	*7.5 per cent. in H_2O.*
Trichloracetic Acid	*10 per cent. in H_2O.*
Gum Ghatti	*0.1 per cent. in H_2O containing a trace of $CHCl_3$ as preservative.*
Magnesium-Standard Solution	*Stock solution prepared by dissolving 10.094 grams of $MgNH_4PO_4.6H_2O$ in 0.1 N.HCl to 1 litre. This contains 1mg of Mg per ml. Working standards containing 1 to 5mg of Mg per 100ml are prepared by diluting the stock solution.*

A volume of 0.1ml of blood, or of blood suitably diluted with distilled water if the magnesium concentration is expected to be high, is washed into 0.5ml of distilled water by means of a wash-out pipette and the mixture is shaken until

haemolysis is complete. Next, 1.1ml of trichloracetic acid is added rapidly, the whole shaken thoroughly, and the mixture is then centrifuged at moderate speed for 10 minutes. 1ml of the supernatant liquid is placed in a 5ml volumetric vessel and treated successively with 1ml of ghum ghatti solution and 1ml of Titan Yellow reagent. The mixture is made up to 5ml with NaOH solution and the whole thoroughly mixed. The optical density is read at 540nm. Blank and standards are prepared in the same manner, 0.1ml of water or working standards being substituted for the blood. The colour develops immediately and is stable for at least three hours at room temperature. Calcium even in concentrations up to 60mg per cent. does not interfere.

Results

In the case in question the post mortem specimens made available to the author contained the following concentrations of Mg^{++} (the female died on the 7th of September of a particular year. Comparative post mortem specimen results are given for the same year) :

	Milligrams of Mg^{++} per 100 millimetres of specimen
Blood from the right chambers of the heart	14·4
Peripheral blood	11·1
Bloody fluid from the uterus	36·8

For the purpose of comparison post mortem bloods from other individuals who died the same year were also examined and the results were :

		Milligrams of Mg^{++} per 100 millimetres of specimen
Peripheral blood (male—died 16th June)		5·0
Heart blood (female—died 25th August)		5·3
Heart blood	(Both from another female—	3·8
Peripheral blood	died 16th September)	4·6

As a still further point of comparison the magnesium concentration of ante-natal venous blood from a live female, aged 24, and approximately 3 months pregnant, was also determined. The results were :

	Milligrams of Mg^{++} per 100 millimetres of specimen
Ante-natal blood from living female— (3 months pregnant. Specimen taken 24th September)	2·0

All these analytical findings indicated that the concentration of 14.4mg per cent. of Mg^{++} in the heart blood, and of 11.1mg per cent. in the peripheral blood of the dead pregnant female were abnormally high concentrations. These figures are lower than fatal, equivalent serum levels quoted by Moore and Wingo but in the absence of air-embolism or other obvious cause of death, it was considered that the high magnesium blood levels found were a responsible factor in this fatality. Idiosyncrasy and shock, more particularly in a pregnant female, may well have provided additional contributory factors. The very high concentration of 36.8mg per cent. of Mg^{++} in the bloodstained fluid from the uterus confirmed that a solution of Epsom Salt has been injected into the organ.

Prior to death the woman was alleged to have said that she felt a sensation of great heat pass through her body. This physiological phenomenon can occur after an injection of magnesium sulphate as was recorded by Spier, Wright and Saylor (1936). These investigators administered intravenous injections of a complex solution containing 42 per cent. of magnesium sulphate and 16 per cent. of calcium gluconate (an antidotal precaution) to volunteers. The injection caused a wave of heat to pass through the body. The arrival of this wave at any location was timed and used to estimate the circulation time of the blood.

Acknowledgments

My thanks are due to Mr. G. E. Bouskell-Wade, Coroner for the City of Leicester, and to Mr. F. L. Cann, the Director of the Home Office Forensic Science Laboratory, Nottingham, for permission to publish this paper.

References

FAWCETT, D. W., *et al.*, 1943, Magnesium sulphate, **123**, 1028.
GARNER, R. J., 1946, *Biochem J.*, **40**, 828.
GREENBERG, D. M., *et al.*, 1933, Magnesium of blood, *J. Biol. Chem.*, **100**, 139.
GREVILLE, G. D., and LEHMANN, H., 1943, *Nature*, Lond., **152**, 81.
HEAGY, F. C., 1948, *Canadian J. Res.*, Section E., **26**, 295.
HIRSCHFELDER, A. D., 1929, Magnesium antagonists, *J.P.E.T.*, **37**, 399.
HIRSCHFELDER, A. D., 1934, Low plasma magnesium, *J.A.M.A.*, **102**, 1138.
HIRSCHFELDER, A. D., 1934, Plasma magnesium in renal insufficiency, *J. Biol. Chem.*, **104**, 647.
JONES, D. I. H., and THOMAS, T. A., 1965, The determination of magnesium in plant material and serum by atomic absorption spectrophotometry, *Hilger Journal*, Vol. IX, No. 3, p. 39.
MOORE, R. M., *et al.*, 1941, Blood levels of magnesium, *Am J. Physiol.*, **133**, 391. Ibid., **135**, 492.
ORANGE, M., and RHEIN, H. C., 1951, *J. Biol. Chem.*, **189**, 379.
SMITH, P. K., *et al.*, 1939, Magnesium, *Am J. Physiol.*, **126**, 720.
SMITH, P. K., *et al.*, 1942, *Review Anaesthesiology*, **3**, 323.
SMITH, P. K., WINKLER, A. W., *et al.*, 1939, Magnesium Distribution, *J. Biol. Chem.*, **129**, 51.
SOLLMANN, T., 1950, *Manual of Pharmacology*, 7th Edition, p. 749.
SPIER, L. C., *et al.*, 1936, Circulation Time Method, *Am. J. Heart*, **12**, 511.
WELT, L. G., and TOSTESON, D. C., 1964, Mode of activation of membrane ATP-ase, by Mg^{++}, *Clin. Res.*, **12**, 73.
WHANG, R., and WELT, L. G., 1963, Observations in Magnesium depletion, *J. Clin. Inves.*, **42**, 305.

Wiener Klinische Wochenschrift
49:241-242, 1936

A Case of Magnesium Sulfate Poisoning

by

Dietrich Roller

From: Ist Medical Clinic of the University of Vienna
(Director: Prof. H. Eppinger)

Magnesium sulfate has been administered parenterally until recently for purposes of narcosis in traumatic tetanus and occasionally also in chorea (1,2). It was applied subcutaneously or intramuscularly in a 25% solution and more recently in intralumbar injection of a 15% solution (3). The intravenous administration of a 3% solution has also been reported to be effective. Recently English authors (4) have included angiospastic, in some cases migraine-like states in magnesium sulfate therapy; these are said to have disappeared upon several intravenous injections of 5 to 10 cc of a 20% magnesium sulfate glucose solution. Schick has recently reported before the Viennese Society for Internal Medicine on a number of cases successfully treated by this method. The following is stated in "Experimentelle Pharmakologie"(5): "If magnesium salts enter the circulation, they have a very toxic effect; administered intravenously, a few decigrams injected at once are enough to cause paralysis of the respiratory centers in larger animals." The large doses of magnesium sulfate used in the treatment of

traumatic tetanus have frequently led to respiratory distress, as has already been reported by Th. Kocher (2). (For detailed literature see 6,7). The English authors and the Viennese speaker have reported no disturbances following injections, nor have harmful effects of magensium sulfate been reported in the above-cited literature similar to that described below.

On June 28, 1935 a 21 year old domestic worker was brought to the hospital by the rescue service with a diagnosis of "spasms following the injection of magnesium sulfate". The patient's face was very flushed, the lips showed a trace of cyanosis. The high respiratory frequency was noted, varying between 50 and 60 and rendering speech difficult. When sitting up she immediately felt dizzy. Her pulse frequency was around 70. Heart and lungs showed no pathological findings. Tendon reflexes were considerably intensified. A further striking feature was the position of the hands and feet. The hands were slightly flexed towards the palms at the wrist joint, the four fingers strongly bent at the base joint and slightly bent at the interphalangeal joint. The thumbs were strongly adducted. The feet were stretched out at the ankle joint and were in a supine position. All these positions were stiffly maintained for a prolonged period until the spasm dissolved. During the attack it was possible to stretch or bend the fingers without causing the patient pain, but left to themselves, they immediately resumed the position described. According to the patient these spasms recurred every few minutes.

The patient told the following: Since her 14th year, allegedly following a purulent frontal sinusitis, she has suffered from severe headaches, which have resisted all treatment. On the day of her admission she received an intravenous injection of a magnesium sulfate solution (2 cc of a 20% solution) from her own physician for this headache. Following the injection she immediately felt dizzy, experienced a feeling of heat, a few seconds later very severe spasms developed in her entire body, particularly the limbs, so that she became quite stiff. She also suffered from severe dyspnea and was dazed to some degree. Since despite the administration of various analeptic agents and 5 cc Afenil her condition did not improve, the patient was transferred to the hospital 2 hours later.

There was no definite Chvostek's sign; a ligature applied to her upper arm in a spasm-free interval yielded a positive Trousseau sign within 45 seconds. This ligature was left in place during the next spontaneous attack and 10 cc calcium Egger (0.7 calcium gluconate, 0.3 calcium levulinate, 10 distilled water) was injected intravenously. The spasms dissolved immediately in the uncongested hand and the two legs, but in the congested hand only when the ligature was removed, after about one minute. Within approximately 10 min the dazed feeling and dizziness also disappeared, the patient felt fully recovered and was released on the following day. It should also be mentioned that the headache which disappeared during the spasms reappeared with the same intensity, according to the patient, following the calcium injection.

The case described is that of a tetany resulting from relative (in relation to the other ions) or absolute calcium deficiency in the serum, but does not reflect damage caused by the magnesium sulfate administered in itself, since the findings typical of magnesium sulfate poisoning (absence of spasms, rapidly developing narcosis, respiratory paralysis) were all absent in this case. This was far more a case of severe interference with the regulatory complex which is only inadequately covered by the term "ionic equilibrium". Proof of this is furnished by the effectiveness of the calcium administered on the tetany.

In a work to be published shortly in Schmiedebergs Archiv (8) a report will be given on severe poisoning following intravenous injection of concentrated sodium salt solutions. The findings made on animals have a great deal of similarity with those cited here. The same treatment was successful in both cases. This shows that the decisive factor in this toxic picture is not the salt in question, but only its high concentration in the blood. This also accords with the fact mentioned in the work referred to above that even highly concentrated solutions have no toxic effect if injected at an appropriately slow speed. Thus the recommendation of this work to proceed with caution when giving intravenous injections of highly concentrated sodium salt solutions is reinforced and may justifiably be extended to the intravenous injection of other highly concentrated salt solutions.

Summary

A 20% intravenous magnesium sulfate solution gives rise to a picture of tetany with respiratory distress which immediately disappears upon intravenous injection of calcium.

Bibliography

See p. 242.

Aus der I. Medizinischen Klinik der Universität Wien
(Vorstand: Prof. H. Eppinger)

Ein Fall von Magnesiumsulfatvergiftung

Von Dietrich Roller

Parenterale Magnesiumsulfatgaben wurden bis vor kurzem nur zur Narkose bei Wundstarrkrampf und gelegentlich auch bei Chorea verabreicht.[1,2] Die Anwendung erfolgte subkutan oder intramuskulär in 25%, in neuerer Zeit auch intralumbal in 15% Lösung.[3] Auch intravenöse Verabreichung einer 3% Lösung soll sich bewährt haben. In letzter Zeit wurden in den Kreis der Magnesiumsulfattherapie durch englische Autoren[4] auch angiospastische, zum Teil migräneartige Zustände einbezogen, die nach mehrmaliger intravenöser Darreichung von 5 bis 10 ccm einer 20% Magnesiumsulfatglukoselösung geschwunden sein sollen. In der Wiener Gesellschaft für innere Medizin hat Schick vor kurzem über eine Reihe mit Erfolg nach dieser Methode behandelter Fälle berichtet. In der „Experimentellen Pharmakologie" heißt es wörtlich:[5] „Kommen Magnesiumsalze in den Kreislauf, so wirken sie sehr giftig; intravenös

genügen bei größeren Tieren schon wenige Dezigramm, auf einmal injiziert, um das Respirationszentrum zu lähmen." Die hohen, bei der Behandlung des Wundstarrkrampfes verwendeten Magnesiumsulfatdosen haben tatsächlich mehrfach zu Atemstörungen geführt, worüber schon Th. Kocher[2] berichtet. (Ausführliche Literatur: [6, 7].) Die englischen Autoren und der Wiener Vortragende haben über keinerlei Störungen im Anschluß an die Injektion berichtet. Aber auch in der vorher genannten Literatur findet sich kein Fall einer schädlichen Magnesiumsulfatwirkung, der dem im folgenden beschriebenen ähnlich ist.

Am 28. Juni 1935 wurde an die Klinik eine 21jährige Hausgehilfin durch die Rettungsgesellschaft unter der Diagnose „Krampfzustände nach Injektion von Magn. sulfur." eingeliefert. Das Gesicht der Patientin war stark gerötet, die Lippen wiesen eine Spur von Zyanose auf. Bemerkenswert schien die hohe Atemfrequenz, die zwischen 50 und 60 schwankte und der Patientin das Sprechen erschwerte. Sie wurde beim Aufsetzen sofort schwindlig. Pulsfrequenz um 70. Herz und Lunge boten keinen pathologischen Befund. Die Sehnenreflexe waren stark gesteigert. Auffallend war ferner die Stellung der Hände und Füße: Die Hände waren im Handgelenk leicht volar flektiert, die vier Finger im Grundgelenk stark, in den Interphalangealgelenken nur leicht gebeugt. Die Daumen waren stark adduziert. Die Füße waren in den Sprunggelenken gestreckt und befanden sich in Supinationsstellung. Alle diese Stellungen wurden durch längere Zeit starr beibehalten, bis sich der Krampf wieder löste. Im Anfall war es wohl möglich, die Finger zu strecken oder zu beugen, ohne dabei der Patientin Schmerzen zu verursachen, doch kehrten sie, sich selbst überlassen, sofort in die geschilderte Stellung zurück. Diese Krämpfe wiederholten sich nach Angabe der Patientin alle paar Minuten.

Die Patientin berichtete: Seit dem 14. Lebensjahr, angeblich im Anschluß an Starkölbadeentzündung, starke Kopfschmerzen, die jeder Therapie trotzten. Dieser Kopfschmerzen wegen bekam sie am Tage der Aufnahme von ihrem Arzt eine intravenöse Injektion einer Magnesiumsulfatlösung (2 ccm einer 20%igen Lösung). Im Anschluß an die Injektion sofort Schwindelgefühl, es wurde ihr heiß, einige Sekunden später sehr starke Krämpfe im ganzen Körper, besonders in den Gliedmaßen, so daß die Patientin ganz steif wurde. Dabei bestand hochgradige Atemnot und eine gewisse Benommenheit. Da sich der Zustand trotz verschiedener Analeptika und 5 ccm Afenil angeblich nicht besserte, wurde die Patientin 2 Stunden später an die Klinik überstellt.

Ein Chvostek-Zeichen war nicht einwandfrei festzustellen, eine am Oberarm im krampffreien Intervall angelegte Staubinde ergab nach 45 Sekunden einen positiven Trousseau. Diese Binde wurde während des nächsten spontan eintretenden Anfalles belassen und in den anderen Arm 10 ccm Kalzium Egger (0·7 Calcium gluconicum, 0·3 Calcium lactobionicum, 10 Aq. dest.) intravenös injiziert. Die Krämpfe in der ungestauten Hand und beiden Beinen ließen augenblicklich nach, in der gestauten Hand aber erst, als etwa eine Minute später die Staubinde abgenommen wurde. Innerhalb 10 Minuten schwand auch Benommenheit und Schwindelgefühl, die Patientin fühlte sich vollkommen geheilt und wurde am nächsten Tage entlassen. — Zu erwähnen ist noch, daß das während der Krämpfe geschwundene Kopfweh nach Angabe der Patientin im Anschluß an die Kalziuminjektion wieder in alter Stärke auftrat.

Der beschriebene Fall stellt eine Tetanie infolge relativen (im Verhältnis zu anderen Ionen) oder absoluten Kalkmangels im Serum dar, aber keine Schädigung durch das verabreichte Magnesiumsulfat an und für sich, da die für Magnesiumsulfatvergiftung typischen Befunde (Krampflosigkeit, rasch einsetzende Narkose, Atemlähmung) hier alle fehlen. Es handelt sich in diesem Fall vielmehr um einen schweren Eingriff in jenen wichtigen Regulationskomplex, den das Wort „Ionengleichgewicht" nur undeutlich umreißt. Der Beweis hierfür liegt in der bekannten Wirksamkeit des verabreichten Kalziums auf die bestehende Tetanie.

In einer demnächst im Schmiedeberg-Archiv erscheinenden Arbeit[8] werden schwere Vergiftungen bei intravenöser Injektion konzentrierter Natriumsalzlösungen beschrieben. Dort am Tier erhobene Befunde haben viel Aehnlichkeit mit den hier erwähnten. Die gleiche Therapie ist hier wie dort erfolgreich. Daraus ergibt sich, daß das Maßgebende für dieses Vergiftungsbild nicht das betreffende Salz, sondern nur seine hohe, im Blut auftretende Konzentration sein kann. Hiermit steht auch die in der genannten Arbeit erwähnte Tatsache in Uebereinstimmung, daß auch sehr konzentrierte Lösungen bei entsprechend langsamer Injektion keine toxischen Wirkungen mehr entfalten. Damit hat die dort gestellte Forderung, Vorsicht bei intravenöser Injektion hochkonzentrierter Natriumsalzlösungen walten zu lassen, eine Erweiterung erfahren und ist mit einiger Berechtigung auch auf die intravenöse Injektion anderer hochkonzentrierter Salzlösungen auszudehnen.

Zusammenfassung. 20% ige intravenöse Magnesiumsulfatinjektion ruft ein tetanisches Zustandsbild mit Atemstörung hervor, das auf intravenöse Kalziuminjektion augenblicklich schwindet.

Literatur: [1] Franke: Zentralbl. f. innere Medizin, 1935. — [2] Kocher, Th.: Dtsch. med. Wochenschr., 1911. — [3] Meyer, H. H. u. Gottlieb, R.: Experimentelle Pharmakologie, 1934. — [4] Pines, N. u. Kieff, M. B.: Magnesium sulphate in the treatment of angiospasms. Lancet, 1933, I, 577. — [5] Siehe bei 3. — [6] Saegesser, Max: Erg. Chir., 1933, Bd. 26. — [7] Nöggerath, C.: Klin. Wochenschr., 1935, 2321. — [8] Lange, F. u. Roller, D.: Ueber die Giftwirkung konzentrierter Salzlösungen. Schmiedeberg-Archiv (im Druck).

Wiener klinische Wochenschrift
64:942-945, 1952

New and Old Data on Tapeworm Cures (Acute Magnesium Sulfate Poisoning upon Oral Administration).

By

Otto A. Rösler

From: IIIrd Medical Service of Graz District Hospital
(Director: Prof. Otto A. Rösler, M.D.).

A few fatal cases have recently come to light following tapeworm cures with aspidium and magnesium sulfate. While the aspidium fatality observed in Germany only occurred on the 17th day following the ingestion of the male fern extract and was caused by an isolated effect on the heart, in our case death took place 7 hours after introducing the magnesium sulfate solution through a duodenal tube, due to central respiratory paralysis with magnesium sulfate narcosis.

An analogous magnesium sulfate fatality in the course of a tapeworm cure with this agent has been reported elsewhere, but unfortunately details are not availbale to us.

These fatalities are such depressing, oppressive occurrences that they provide sufficient reason for a statement on this problem, particularly if we are able to look back on the decades of experience of a hospital as large as the Graz District Hospital. During this time more interest and attention has been devoted to tapeworm carriers than usually appears to be the case.

Among tapeworms that infest the human intestine in our latitudes Taenia saginata, Taenia solium and Bothriocephalus latus occur for the most part and man represents the principal host for these, although in the case of Taenia solium he may also become the intermediate host (Cysticercosis).

In this country we have mainly to do with Taenia saginata, the bovine tapeworm, which presumably may be attributed to the fact that the hog infested with cysticercus, owing to its extreme prevalence, cannot as readily evade the meat inspector as the more rarely infested beef. Due to the resulting meat inspection and the consequent elimination of the hog with cysticercosis, Taenius solium has become a rarity in Styria and one can almost claim that this species of Taenia has died out in Austria. According to the literature, the same applies to West Germany. This is supported by the fact that this species of Taenia did not increase even during the period of black market slaughtering, and has not even now, in places in which there is virtually no inspection of hogs when the animals are slaughtered at home. Borthriocephalus latus has only been observed in Styria in prisoners of war or persons returning home from northern waters or who were forced by war circumstances to spend some time in these areas during the war or post-war period.

I myself had occasion to purge Bothriocephalus latus in the First World War from a Russian prisoner of war suffering from a condition resembling extremely severe pernicious anemia,

and this time from a person who returned from Finland suffering from anemia. Thus the bulk of our tapeworms is made up of bovine tapeworm (Taenia saginata). This species is very common at any rate in Graz, so that we have had occasion to see and treat several hundred Taenia carriers in the course of the past four decades and gained a considerable amount of experience.

First of all I should like to state as a preliminary that we determine for ourselves whether the patient requesting a tapeworm treatment is actually a tapeworm carrier, which is by no means invariably the case. One may be dealing with a case of self-deception on the part of the patient, or with a deliberate desire to deceive the physician for a specific reason. Meat fibers, tendinous remnants from meat dishes, plant fibers, etc. may give the layman the impression of being tapeworm joints, or else female patients may have an entirely different aim in undergoing a tapeworm cure and may bring a tapeworm joint hardened in alcohol, but this does not belong to her and is brought in to mislead. Such female patients are generally very indignant when no credence is given their alcohol preparation and the cure is not immediately instituted, but instead the decision is left to the detection of tapeworm in the stools or the enema fluid of the patient herself. This has occurred again and again and the deception has sometimes succeeded in the case of inexperienced and credulous younger physicians. Furthermore it is a basic rule that no tapeworm cure is carried out outside the hospital and there only when we are able to be present during the cure and can

be reached at all times. We decline all requests for tapeworm cures in the case of delicate children, weak or ill individuals, weak old persons, in cases of circulatory decompensation and advanced pregnancy, since the procedure is fairly violent and not without danger, but we are not as hesitant or cautious in the case of kidney or liver patients or diabetics, provided aspidium is not used as purgative, to which I shall return later. In the course of our long period of experience we have tested virtually every tapeworm agent and until a few years ago, because of unsatisfactory results, we always came back to the fern extract. The combination of an aspidium cure with 4.0 chloroform + 160 castor oil has often been used without incident. Success with aspidium and this combination was still the best, even though quite unsatisfactory, quite aside from the fact that when using fern extract the whole day was spent holding the breath, and nausea, vomiting and collapse could scarcely be avoided, not to mention more severe complications such as icterus, amblyopia, amaurosis, tetanic spasms and death. There was always severe disappointment when in spite of this very disagreable procedure and in spite of the less severe complications referred to above the patient had to be told all too often that the head had not been passed and the cure would have to be repeated in 8 to 12 weeks, as soon as new segments were passed. We have used aspidium extract in all possible forms: fluid in capsules, intubated into the duodenum and in various pharmaceutical preparations in adequate dosages, but were not able to improve

the success of the purgative treatment appreciably. It was also impossible to obtain fresh fern extract. Based on the results obtained we are forced to doubt that there is any difference in the effect of the extract whether fresh or old, quite aside from the fact that there was no possibility of controlling whether the extract was fresh or old. We were very glad when we were able to turn to atebrin, Carlsbad salts (Högler) and Epsom salts. After suitable preparation, in which the intestine, particularly the colon, was cleansed of old fecal masses, to allow the worm free passage upon purging, 0.8 g atebrin was administered fasting per os and the patient was kept in bed. Four hours later the patient received 30 g magnesium sulfate in 200 cc water and this was repeated every 2 hours if no evacuation had taken place by that time. The head was passed in 33% of cases and this result did not improve even when Carlsbad salts were used as purgative instead of magnesium sulfate. The worm showed yellow discoloration, just as persons with malaria treated for prolonged periods with atebrin have a yellow skin color. This yellow color is mainly due to a fairly strong absorption of atebrin and not to imbibition of bile pigments or any other toxic effect. In general atebrin appears to have very little toxicity. We observed no nausea or any other disagreable side effect.

Because of the unsatisfactory result we next tried our luck with Högler's Carlsbad salt tapeworm cure with the following modification:

After establishing that mature proglottides were being passed or that in the recent past not very large pieces of tapeworm had been passed either spontaneously or following some procedure, we carried out a preliminary cure consisting, as mentioned before, of cleansing the intestine, and particularly the colon, of all fecal masses and limiting meals for 2 days to low-bulk foods such as a soft diet or tea. On the eve of the cure a sedative was administered and the next morning, on a fasting stomach, the duodenal tube was introduced under radiological guidance. When the tube was in a good position in the duodenum it was pushed in further by 5 to 10 cm, so that the purgative should not find its way into the stomach. Before instilling the purgative we rapidly gave an intravenous sodium chloride infusion (500 cc 0.9% NaCl) with analeptics (2 cc Coramine ±1 cc 20% caffeine sodium benzoate). Next the lukewarm Carlsbad salt solution was instilled in a quantity of 80 to 120g according go the patient's own point of view, in 250 cc water. For this we used two 20 cc syringes so as to shorten the time of instillation as far as possible. By this means on the one hand crystallization of the salt can be avoided and on the other the highly concentrated salt solution can act on the tape worm in its entire dose. Within a very short time, in most cases 20 to 30 min, this leads to diarrhea and evacuation of the tapeworm, including the head. In order to anticipate possible nausea or vomiting, a strong dose of Nautisan is administered at the proper time

before the cure. To deal with a possible collapse an entire circulatory support apparatus is kept in readiness. So far we have achieved the best results with this cure, provided the duodenal tube was correctly positioned, which is a basic condition of the method. Indisputable advantages are as follows: first of all the entire procedure is over within a very short time, secondly the worm, including the head, is generally passed in the first cure and thirdly the worm carrier is not exposed to complications and danger as is the case with fern extract, and, according to recent experience, when Epsom salts are used instead of Carlsbad salts. This cure can be repeated at any time without danger and may also be used without reservations in diabetics and patients with liver and kidney diseases.

Only the general contraindications for any tape worm cure referred to at the beginning should be taken into account. Thus the Carlsbad salt tapeworm cure may be regarded as the method of choice and emphatically recommended. Occasionally we have also modified this tapeworm cure by using magnesium sulfate (Epsom salts) in a dose of 80 to 120 g dissolved in 200 cc water intubated into the duodenum, instead of Carlsbad salts. In 6 out of 12 cases, in other words 50%, the tapeworm was evacuated. The last of these cases died tragically of central respiratory paralysis with signs of very severe magnesium narcosis, which came as a considerable surprise, since the previous 11 cases had shown no signs of toxic effect on the central nervous system and we knew from the literature

that practically the same quantity of a 20% magnesium sulfate solution had been used every other day in severe cases of jaundice with very good results.

Since the occurrence, to our knowledge, of one other fatality resulting from a tapeworm cure with magnesium sulfate, we have completely abandoned the use of magnesium sulfate and most strictly advise and warn against the peroral use of large doses of Epsom salts. In the literature available to me I found not a single case in which the peroral administration of Epsom salts had led to such lethal poisoning and Rudolf Schmidt in his "Therapie und Prophylaxe innerer Krankheiten" [Therapy and prophylaxis of internal diseases], 1948, writes that the narcosis and paralysis observed in animals (rabbit, calf) after the peroral administration of large doses of magnesium sulfate had no analogy in humans. Neither in the work of Meyer-Gottlieb nor in Møller's Pharmacology, nor in the "Handbook of Experimental Pharmacology" Supplement X is there any indication of any magnesium sulfate poisoning after peroral administration. Starkenstein has referred to the strong purgative effect of orally administered magnesium salts and has stressed that consequently its absorption becomes less with increasing dosage. However, as is demonstrated by our own case, there are conditions under which absorption from the intestinal tract is such that the entire body is inundated with large quantities of magnesium ions, which can lead to deep narcosis and paralysis of the respiratory center

with this mode of administration, just as it had been observed upon parenteral application. In Møller's "Pharmacology" a patient is described who was mistakenly treated intravenously with magnesium sulfate instead of sodium sulfate and died. In our case the fresh lungs contained more than three times the magnesium of a normal fresh lung according to the Medical-Chemical Institute of Graz University (Director: Prof. H. Lieb, M.D.), as copied from judicial records. According to a forensic medical pathologist (Dr. M. Fossel) the excessive absorption of Epsom salts from the intestine was promoted by extensive areas of adhesions of the abdominal organs, discovered upon opening the cadaver, particularly in the upper abdomen. The obstruction of free passage of the loops of the small intestine caused thereby is believed to have been the cause of Epsom salt solution remaining in the small intestine for longer periods and of the increased absorption of magnesium sulfate.

Having found no case of acute, fatal magnesium sulfate poisoning following peroral administration in the literature available to me, I believe a brief description of this case and of the autopsy findings may be of some interest;

H.H., 62 year old woman, slim build, weakly developed musculature and moderate nutritional state.

After the preliminary measures described above the following procedure was carried out with the patient fasting:

At 11 a.m. 200 cc 40% magnesium sulfate ($MgSO_4$) and 50 cc 20% $MgSO_4$ (i.e. a total of 90 g $MgSO_4$) was infused with 250 cc water. At the same time 0.10 caffeine sodium benzoate + 0.001 strychnine nitrate was administered subcutaneously. After a few minutes slight nausea, no vomiting.

At 11:15 a.m. slight collapse with small, frequent pulse, drop in blood pressure and moderate outbreak of sweating, which subsided after administration of analeptics. Urgent evacuation of stools. Roughly 1 liter liquid stools were evacuated into the bedpan with a few worm segments.

11:20 a.m. Patient complained of dyspnea, Lobelin was ineffective. Cyanosis and paleness rapidly increased. Peripheral and central analeptics administered intravenously. Because of progressive dyspnea and increasingly shallow respiration with bradycardia, pulse frequency of 60 and slight arrhythmia, artificial respiration was begun. The patient rapidly entered a comatose state with moderately wide pupils that still reacted to light, the corneal reflexes were weak. Limbs slack.

12 a.m. The most marked manifestation was the dyspnea, which temporarily improved under artificial respiration, Lobelin and analeptics. Blood sugar normal. To counteract the increasingly apparent magnesium effect, 10% calcium gluconate was repeatedly administered intravenously. Continuous infusion of cardiac and vasoactive agents. The sucking off of mucous masses from the trachea and evacuation

of the stomach gradually improved the patient's condition, respiration became increasingly deeper and more regular, apparently due to improvement of the Mg/Ca quotient upon the effect of the calcium injections.

Towards 3 p.m. the patient woke from the coma, became responsive, the cyanosis subsided, heart action and respiration were satisfactory. The patient drank some liquids, felt weak but was essentially free of symptoms.

Unfortunately the improvement did not last long. At 4 p.m. respiration became shallow and slower, the patient collapsed again, became comatose and from 5 p.m. no longer responded. Despite continued artificial respiration, Lobelin, Coramine, circulatory and cardiac active agents as well as large doses of calcium gluconate and continuous infusion of physiological saline, her condition visibly deteriorated and she died at 17:20 p.m. with a picture of central respiratory paralysis and cardiac and circulatory weakness.

The autopsy at the Forensic Medical Institute of Graz University carried out by Dr. M. Fossel revealed the following, given here in abstract form:*

Opening of the body revealed a marked dessication and thickening of the blood in the entire body, with simultaneous plethora in all internal organs. There was also an immense

---

* I thank the authorities of the Forensic Medical Institute of Graz University (Director at the time: Dr. M. Fossel) for furnishing the autopsy findings and attendant files.

dilatation of the left ventricle of the heart with stripy extravasation in the inner wall and a massive accumulation of blood and fluid in the lungs (pulmonary edema). A tapeworm was discovered in the subject's small intestine. The loops of the small intestine were slightly red with vessels abundantly filled with blood. In the upper abdomen there were areas and strands of adhesions between the greater omentum and the anterior abdominal wall, as well as between the liver and diaphragm. There was c. one scoopful (100 cc) of clear yellowish fluid in the small pelvis. On the cut surface of the lung, the lung tissue was strikingly filled with blood and fluid, particularly in segments with an outwardly dark appearance. Upon pressure a very large amount of dark red foamy fluid issued from the cut surface; the same fluid also issued from the severed bronchi. The heart corresponded in size to the body's fist, the pericardium was delicate and free of hemorrhages. The apex of the heart was made up of both ventricles. The right ventricle was slightly dilated, containing an abundance of coagulated blood with a dark red and yellowish gleam, the left ventricle in cross section was considerably dilated, forming a hemispherically rounded apex. On the inner wall of the left ventricle there were several punctiform, dark red hemorrhages, particularly in the area of the papillary muscles. Just before the pulmonary valves on the inner wall of the right ventricle there was an ecchymosis the size of a fairly large lentil. The heart valves themselves were all delicate and capable of closure. The coronary artery branches were of medium width, freely penetrable, showing only slight

yellowish deposits. The mucosa of the entry to the larynx was in loose folds and somewhat gelatinous. Spleen, liver and stomach were normal. The mucosa of the small intestine was somewhat red, the large intestine contained a fairly abundant quantity of yellowish-green, thinly mucous mass. The mucosa of the large intestine was normal. Adrenals and bladder were normal.

The cranial vault was fairly thick, the dura mater stretched, the pia mater delicate, transparent, with a moderate amount of blood, the brain convolutions very narrow, particularly in the region of the occiput. Convolutions and sulci were distinct, the cut surface of the brain revealing distinct tracings, with no increase in blood or fluid quantities. The cut surface of the brain tended to be dry. The median portions of the cerebellum somewhat pressed towards the occipital foramen. The findings of the autopsy would have had no explanation without knowledge of the patient's history. According to her history, death took place due to respiratory paralysis in the form of magnesium sulfate narcosis, caused by excessive entry of perorally administered magnesium sulfate into the circulation. The tragic course reported in the history immediately following the worm cure may be regarded as proof that this assumption is correct."

As has already been mentioned, another fatal case has been observed following the peroral administration of Epsom salts in addition to this magnesium sulfate death, and quite recently a third fatality following a tapeworm cure has been reported

in Medizinische Klinik 19, 1952 caused by aspidium, reported by H. Greiner of the Institute of Forensic Medicine of the Düsseldorf Medical Academy, in which an isolated effect on the heart was the most striking feature.

Thus we are dealing with three fatalitis following tape worm cures caused by the toxic effect of the purgative used. It has long been known concerning the active principle of aspidium, i.e. amorphous filicic acid = filicin that in some circumstances it can lead to extremely severe complications and even death, sometimes even within a few hours. In itself filicin is slowly and incompletely absorbed, but if in certain circumstances large quantities are absorbed, toxic manifestations of varying intensity may appear. The simultaneous consumption of alcohol enhances absorption and is therefore contraindicated. The therapeutic success of the tapeworm cure is probably due to the muscle paralyzing effect of filicin on lower animals. Thus while it has long been known that aspidium preparations may occasionally have a deadly effect, it is an entirely new observation that orally administered magnesium sulfate can also cause death when optimally absorbed.

In his "Pharmacology", Møller has written that magnesium salts administered per os are absorbed slowly and very incompletely, which explains the therapeutic use of these substances as purgatives. If, however, large doses are repeatedly administered to patients with impaired renal function, a rise in the magnesium concentration in the plasma is observed

to 10-11 mg%, which results in a comatose state. It is also claimed that the repeated administration of purgative doses of magnesium sulfate in patients with nephritis can cause a coma very similar to a uremic coma, which is understandable if one knows that subcutaneously or intramuscularly injected magnesium salts are rapidly absorbed and the greater part quickly excreted, mainly through the kidneys. It is also known that complete narcosis, with unconsciouceness so profound that an operation could be performed can also take place in humans upon parenteral administration of magnesium salts. Another known fact is that magnesium ions have a distinct curare effect, so that it is not possible to elicit muscular contractions by nerve stimulation. Because of this selective effect, magnesium was and is the principal and indispensable agent in the treatment and relaxation of tetanic muscle spasms in tetanus. However, the therapeutic range of action for deep magnesium narcosis is very narrow, because the dose required to produce narcosis is very close to the dose that gives rise to respiratory paralysis and therefore respiratory disturbances may develop as well as the desired muscular paralysis. It is also known that the parenteral administration of magnesium salts causes light (? poor copy) narcosis in a number of experimental animals even in a plasma concentration of c. 9 to 10 mg%; at a concentration of 15 to 18 mg% there is deep, reflexless narcosis and at around 20 mg% respiratory paralysis sets in. In our case the magnesium content of the fresh lung per 100 g tissue was 24.5 and 25.5 mg%, in other words more than three times that of a normal fresh lung,

a finding which suggests the correctness of the assumption that in this case a large part of the orally administered magnesium was absorbed and thus caused the narcosis and central respiratory paralysis. In view of the fact that the magnesium-calcium antagonism was so heavily weighted in favor of magnesium, we can also understand why the calcium injected as counter agent was only initially capable of producing brief improvement, but later proved entirely ineffective.

In summary it may be stated that both aspidium and magnesium sulfate, even when administered orally, harbor such dangerous possibilities that no tapeworm carrier should be exposed to these dangers. The bovine tapeworm most prevalent in our latitudes is so marhmless a companion that it would be irresponsible to expose the tapeworm carrier to the danger of death for its sake, particularly since we have in the Carlsband salt tapeworm cure such a safe and well tried method at our disposal. When the contraindications referred to exist, we always have the possibility of expelling the greater part of the tapeworm by means of a powerful purgative, after which the tapeworm carrier need not expect any disturbance for c. 2 to 3 months, until the worm has grown again. In response to likely objections that aspidium extremely rarely causes fatalities it must be said that this is only because filicin is absorbed slowly and incompletely. If, however, conditions of absorption for some unpredictable reason were to be extremely favorable, the toxic and possibly lethal dose would be reached and a case would arise which in view of the other possibilities that exist should be avoided at all costs.

Bibliography

See p. 945.

Aus der III. Medizinischen Abteilung des
Landeskrankenhauses Graz
(Vorstand: Hofrat Univ.-Prof. Dr. med. Otto A. Rüster)

Neues und Altes über die Bandwurmkuren

(Zugleich ein Beitrag zur akuten Magnesium sulfuricum-Vergiftung bei oraler Verabreichung dieses Mittels)

Von Otto A. Rüster

In letzter Zeit wurden einige Todesfälle bekannt, die im Gefolge durchgeführter Bandwurmkuren mit Filix mas und Magnesium sulfuricum eintraten. [illegible] im Deutschen Reich [illegible] Filix [illegible] am 17. Tage nach der [illegible] durch isolierte Bewegung [illegible] Fall der Eaton [illegible]

[illegible]

Diese [illegible] so [illegible] und [illegible] Verlaufsweise, daß sie [illegible] zu einer [illegible] auf [illegible] [illegible] wie es [illegible] [illegible] als [illegible] zu [illegible]

[illegible] wir es [illegible] dem [illegible] [illegible]

wo die Fleischbeschau bei der Mehrzahl der Hausschlachtungen der Schweine zumeist ganz erfolglos in Verwendung erfahren hat. Den Bothriocephalus latus sah man in der Steiermark nur bei Kriegsgefangenen und Heimkehrern, die von den nordischen Gewässern stammten oder während des Krieges und der Nachkriegszeit infolge des Kriegsgeschehens sich in diesen Zonen aufhalten mußten.

Auch ich konnte den Bothriocephalus latus im ersten Weltkrieg bei einem an schwerster perniciosaähnlicher Anämie leidenden russischen Kriegsgefangenen und heuer bei einem Heimkehrer aus Finnland, der keine Anämie hatte, abtreiben. Das Gros unserer Bandwürmer stellt also der Rinderbandwurm (Taenia saginata) dar. [illegible] Graz alles [illegible] [illegible] zu sehen und zu behandeln [illegible] dabei auch manches [illegible]

Zuerst möchte ich gleich [illegible] festlegen, daß wir uns selbst [illegible] [illegible] dabei nur eine [illegible] [illegible] will [illegible]

[illegible]

[illegible] ob frisch oder alt, zweifelnd, ganz abgesehen davon, daß man [illegible] keine Kontrollmöglichkeit hatte, feststellen, ob der Extrakt frisch oder alt war. Wir waren schon recht frech, als wir uns dem Atebrin, dem Karlsbadersalz (Högler) und dem Bittersalz zuwenden konnten. Wir haben nach entsprechender Vorbereitung, wobei der Darm, speziell der Dickdarm, von älteren Fäkalmassen gereinigt wurde, damit der Wurm bei der Abtreibung möglichst freie Passage habe, auf nüchternen Magen 0·8 g Atebrin per os verabreicht und den Kranken anschließend im Bett gehalten. 4 Stunden später gaben wir 30 g Magnesium sulfuricum in 200 ccm Wasser und wiederholten diese Gabe nach 2 Stunden, wenn bis zu diesem Zeitpunkt keine Entleerung erfolgt ist. Der Kopf ging in 35% der Fälle ab, und dieses Ergebnis besserte sich auch nicht, wenn wir als Abführmittel das Magnesium sulfuricum durch Karlsbader Salz ersetzten. Der Wurm war ganz gelb verfärbt, wie auch durch längere Zeit atebrinbehandelte Malariker eine Gelbfärbung der Haut zeigten. Diese Gelbfärbung beruht hauptsächlich auf stärkerer Absorption des Atebrins und nicht auf Gallenfarbstoffimbibition oder einer anderen toxischen Wirkung. Das Atebrin scheint überhaupt sehr wenig toxisch zu sein, wir sahen nie Uebelkeit oder andere unangenehme Nebenerscheinungen.

Wegen des unbefriedigenden Erfolges versuchten wir dann unser Glück mit der Höglerschen Karlsbadersalz-Bandwurmkur und führten diese in nachfolgender Modifikation durch:

Nach der Feststellung, daß reife Proglottiden abgehen oder in letzter Zeit keine alten großen Stücke eines Bandwurmes spontan oder im Gefolge irgend einer Maßnahme abgegangen seien, machen wir die Vorbereitungskur, die darin besteht, daß wir, wie schon erwähnt, den Darm, speziell das Colon, von alten Stuhlmassen reinigen und die Mahlzeiten 2 Tage lang nur auf schlackenarme Speisen, wie Breikost und Teetage, beschränken. Am Vorabend geben wir ein Sedativum und führen nächsten Morgen bei nüchternem Magen die Duodenalsonde unter Röntgenkontrolle ein. Wenn die Sonde im Duodenum gut sitzt, schieben wir sie noch um 5 bis 10 cm tiefer ein, damit das Abtreibemittel ja nicht in den Magen gelangt. Bevor wir nun das Abtreibemittel instillieren, machen wir rasch eine intravenöse Kochsalzinfusion (500 ccm 0·9% NaCl) mit Analeptica (2 ccm Coramin + 1 ccm 20% Coff. na. benz.). Anschließend erfolgt die Instillation der lauwarmen Karlsbadersalzlösung, je nach der Verfassung des Kranken 80 bis 120 g Karlsbadersalz (Sal. carol. factitium) in 250 ccm Wasser. Wir verwenden dazu 2 Stück 20-ccm-Spritzen, um die Zeit der Instillation möglichst abzukürzen, womit einerseits die Kristallisation des Salzes vermieden wird und anderseits diese hochkonzentrierte Salzlösung in der gesamten Dosis auf den Bandwurm einwirken kann, was dann innerhalb kürzester Zeit, zumeist in 20 bis 30 Minuten, zum Durchfall und Abgang des Bandwurmes samt dem Kopf führt. Um dem eventuellen Brechreiz und Erbrechen vorzugreifen, geben wir zeitgerecht vor der Kur Nautisan dosis fortior. Zur Bekämpfung eines eventuellen Kollapses steht der ganze Kreislaufstützungsapparat bereit. Mit dieser Kur haben wir bei gutem Sitz der Duodenalsonde, was eine Grundbedingung ist, bisher die besten Erfolge erzielt. Die unbestreitbaren Vorzüge sind: erstens ist die ganze Prozedur in kürzester Zeit erledigt, zweitens geht der Wurm samt dem Kopf zumeist bei der ersten Kur ab und drittens setzt man den Wurmträger keinen Komplikationen und Gefahren aus, wie das beim Farnkrautextrakt und nach neuesten Erfahrungen auch bei Verwendung des Bittersalzes statt des Karlsbadersalzes der Fall ist. Diese Kur kann jederzeit gefahrlos wiederholt werden und kann auch bei Zuckerkranken, Leber- und Nierenkranken ohne Bedenken herangezogen werden.

Nur die eingangs erwähnten allgemeinen Kontraindikationen gegen jede Bandwurmkur sind auch hier zu berücksichtigen und nicht außer acht zu lassen. Somit kann die Karlsbadersalz-Bandwurmkur als Methode der Wahl bezeichnet und bestens empfohlen werden. Wir haben zwischendurch diese Bandwurmkur auch so modifiziert, daß wir statt des Karlsbadersalzes Magnesium sulfuricum (Bittersalz) in der Dosis von 80 bis 120 g in 200 ccm Wasser gelöst transduodenal eingeflößt haben. Bei 12 Fällen ging der Bandwurm in 6 Fällen, also in 50% ab; der letzte dieser Fälle ging tragischerweise unter den Zeichen schwerster Magnesiumnarkose an zentraler Atemlähmung letal aus, was sehr überraschend kam, nachdem die vorausgegangenen 11 Fälle keinerlei Zeichen einer toxischen Wirkung auf das Zentralnervensystem zeigten und wir aus der Literatur wußten, daß andernorts bei schweren Fällen von Gelbsucht nahezu die gleichen Quantitäten einer 20%igen Magnesiumsulfatlösung jeden 2. Tag mit bestem Erfolg angewendet wurden.

Nachdem sich, wie wir wissen, an anderer Stelle bei einer ebenfalls mit Magnesium sulfuricum durchgeführten Bandwurmkur noch ein Todesfall ereignet hat, haben wir das Magnesium sulfuricum gänzlich ausgeschaltet und müssen auch von der peroralen Verwendung so großer Dosen von Bittersalz strikteste abraten und nachdrücklichst warnen. Ich fand in der mir zugänglichen Literatur keinen einzigen Fall, bei dem die perorale Verabreichung von Bittersalz zu solcher letaler Vergiftung geführt hätte, und Rudolf Schmidt schreibt in seiner Therapie und Prophylaxe innerer Krankheiten vom Jahre 1948, daß die beim Tier (Kaninchen, Kalb) zu beobachtende Narkose und Lähmung nach peroraler Zufuhr entsprechend großer Dosen von Magnesium sulfuricum beim Menschen kein Analogon habe. Weder bei Meyer-Gottlieb noch in der Pharmakologie von Møller und im Handbuch der experimentellen Pharmakologie, Ergänzungsband X, finden sich Anhaltspunkte über Magnesium sulfuricum-Vergiftungen bei peroraler Verabreichung. Starkenstein weist auf die stark abführende Wirkung der oral verabreichten Magnesiumsalze hin und betont, daß demzufolge die Resorption derselben mit zunehmender Größe der Dosis immer kleiner wird. Es muß aber, wie unser Fall zeigt, doch Bedingungen geben, unter welchen die Resorption auch vom Darmkanal so optimal ist, daß der ganze Körper mit großen Mengen von Magnesiumionen überschwemmt wird, die auch bei dieser Verabreichungsart zu tiefer Narkose und Lähmung des Atemzentrums führen können, wie es bei parenteraler Applikation bekannt ist. In der Pharmakologie von Møller wird ein Patient angeführt, der statt mit Natriumsulfat irrtümlich mit Magnesiumsulfat intravenös behandelt wurde und gestorben ist. In unserem Fall enthielt die frische Lunge mehr als das Dreifache an Magnesium einer normalen frischen Lunge laut dem Gutachten des Medizinisch-Chemischen Institutes der Universität Graz (Vorstand: Prof. Dr. H. Lieb), Abschrift aus dem Gerichtsakt. Für die Begünstigung der so überreichlichen Resorption des Bittersalzes aus dem Darm führt der gerichtsmedizinische Obduzent (Doz. Dr. M. Fossel) die bei der Leichenöffnung gefundenen ausgedehnten flächenhaften Verwachsungen der Bauchorgane untereinander, vor allem im Oberbauch an. Die dadurch bedingte Behinderung der freien Beweglichkeit der Dünndarmschlingen wird die Ursache längeren Verweilens der Bittersalzlösung im Dünndarm und für die vermehrte Resorption des Magnesiumsulfates ausschlaggebend gewesen sein.

Nachdem sich in der mir zugänglichen Literatur kein Fall einer akuten, tödlich verlaufenden Magnesium sulfuricum-Vergiftung nach peroraler Verabreichung des Mittels vorfand, erscheint mir die kurz gefaßte Beschreibung dieses Falles und des Obduktionsbefundes nicht ohne Interesse zu sein:

H. H., 62jährige Frau, von schlankem Körperbau, schwach entwickelter Muskulatur und mäßig reduziertem Ernährungszustand.

Nach der oben beschriebenen Vorbereitungskur werden auf nüchternen Magen um

11 Uhr 200 ccm einer 10%igen Magn. sulf. ($MgSO_4$) und 50 ccm einer 20%igen $MgSO_4$ (also insgesamt 90 g $MgSO_4$) auf 250 ccm Wasser eingeflößt. Gleichzeitig wird Coff. n. b. 0·15, Strych. nitric. 0·001 subkutan gegeben. Nach einigen Minuten leichte Uebelkeit, kein Erbrechen.

11.15 Uhr: Leichter Kollaps mit kleinem frequentem Puls, Blutdrucksenkung und mäßiger Schweißausbruch, der sich nach Verabreichung von Analeptica wieder bessert. Abgang von Stuhl unter lebhaftem Drang. Es fand sich ungefähr 1 Liter flüssiger Stuhl mit einigen Wurmgliedern in der Leibschüssel.

11.20 Uhr: Klage über Atemnot, Lobelin ohne Wirkung. Zyanose und Blässe nehmen rasch zu. Periphere und zentrale Analeptika intravenös. Wegen zunehmender Atemnot und Verflachung der Atmung bei Bradykardie, Pulsfrequenz 60 und leichter Arrhythmie wird künstliche Atmung eingeleitet. Rasch entwickelt sich ein komatöses Zustandsbild mit mittelweiten, auf Licht noch reagierenden Pupillen, Kornealreflex schlecht auslösbar. Extremitäten schlaff.

12 Uhr: Im Vordergrund der Erscheinungen steht die Atemnot, die sich auf künstliche Atmung, Lobelin und Analeptika vorübergehend bessert. Blutzucker zeigt normale Werte. Als Gegenmittel gegen die nun immer offensichtlicher werdende Magnesiumwirkung wird wiederholt 10%iges Calcium gluconicum intravenös verabreicht. Dauertropfinfusion mit Herz- und Gefäßmitteln. Absaugen schleimiger Massen aus der Trachea und Entleerung des Magens bessern den Zustand allmählich, die Atmung wird anscheinend durch die Besserung des Mg Ca-Quotienten unter der Einwirkung der Kalziuminjektionen zunehmend tiefer und regelmäßiger.

Gegen 15 Uhr erwacht Patientin aus dem Koma, wird ansprechbar, die Zyanose geht zurück, Herztätigkeit und Atmung zufriedenstellend. Patientin nimmt Flüssigkeit zu sich, fühlt sich zwar schwach, ist aber im wesentlichen beschwerdefrei.

Die Besserung hält aber leider nicht lange an, um 16 Uhr verflacht sich die Atmung, sie wird langsamer und die Kranke verfällt wieder, wird komatös und ist ab 17 Uhr nicht mehr ansprechbar. Trotz fortgesetzter künstlicher Atmung, Lobelin, Coramin, Kreislauf- und Herzmitteln sowie großen Dosen von Calcium gluconicum und der Dauertropfinfusion mit physiologischer Kochsalzlösung verschlechtert sich der Zustand zusehends und um 19.20 Uhr tritt unter dem Bilde zentraler Atemlähmung sowie Herz- und Kreislaufschwäche der Exitus letalis ein.

Die Obduktion im Gerichtlich-Medizinischen Institut der Universität Graz, durchgeführt von Doz. Dr. M. Fossel ergab auszugsweise:*

„Die Leichenöffnung ergab eine auffallende Austrocknung und Eindickung in der gesamten Leiche bei gleichzeitiger Blutfülle aller inneren Organe. Es fand sich weiter eine mächtige Ausweitung der linken Herzkammer mit streifenförmigen Blutunterlaufungen ihrer Innenwand und eine mächtige Blut- und Flüssigkeitsanschoppung der Lungen (Lungenödem). Im Dünndarm der Frau wurde ein Bandwurm festgestellt. Die Dünndarmschlingen sind leicht gerötet mit stark blutgefüllten Gefäßen. Im Oberbauch finden sich etwas flächenhafte und strangförmige Verwachsungen zwischen großem Netz und vorderer Bauchwand und zwischen Leber und Zwerchfell. In kleinen Becken etwa 1 Schöpflöffel voll (100 ccm) klarer gelblicher Flüssigkeit. An der Lungenschnittfläche ist das Lungengewebe vor allem in den schon außen dunkler gefärbten Abschnitten auffallend blut- und flüssigkeitsreich, von der Schnittfläche entleert sich auf Druck sehr reichlich dunkelrote, schaumige Flüssigkeit; die gleiche Flüssigkeit entleert sich auch aus den durchgeschnittenen Bronchien. Das Herz entspricht an Größe der Leichenfaust, das Herzfett ist zart, frei von Blutungen. Die Herzspitze wird von beiden Kammern gebildet, die rechte Herzkammer ist geringfügig ausgeweitet, sie enthält reichlich dunkelrote und gelblich durchscheinende Blutgerinnsel, die linke Herzkammer ist im Querschnitt stark ausgeweitet mit halbkugelig abgerundeter Herzspitze. An der Innenwand der linken Herzkammer finden sich mehrere punktförmige dunkelrote Blutungen, vor allem im Bereich der Papillarmuskeln. Auch knapp vor den Pulmonalklappen an der Innenwand der rechten Herzkammer eine gut Linsengroße Blutunterlaufung. Die Herzklappen selbst sind durchwegs zart und schlußfähig, die Kranzschlagadern des Herzens sind mittelweit, frei durchgängig und zeigen nur geringfügige gelbliche Einlagerungen. Die Schleimhaut am Kehlkopfeingang in stark lockere Falten gelegt und etwas sulzig aufgelockert. Milz, Leber und Magen o. B. Die Schleimhaut des Dünndarmes ist geringfügig gerötet, der Dickdarm enthält mäßig reichlich gelblichgrüne dünnschleimige Massen, die Schleimhaut des Dickdarmes zeigt keine Auffälligkeiten. Nebennieren, Nieren und Harnblase o. B.

Das Schädeldach ziemlich dick, die harte Hirnhaut gespannt, die weichen Hirnhäute zart, durchscheinend, mäßig blutreich, die Hirnwindungen vor allem im Hinterhauptsbereich sehr schmal. Windungen und Furchen deutlich ausgeprägt, an der Schnittfläche zeigt das Gehirn deutliche Zeichnung, keinen erhöhten Blut- und Flüssigkeitsgehalt. Die Schnittfläche des Gehirns ist eher trocken. Die mittleren Anteile des Kleinhirns etwas in das große Hinterhauptsloch eingepreßt. Der bei der Leichenöffnung festgestellte Befund wäre ohne genauere Kenntnis der Vorgeschichte unverständlich. Auf Grund der Vorgeschichte ist der Tod der Patientin infolge einer Atemlähmung eingetreten, welche durch einen übermäßig großen Uebertritt von rasch verabreichtem Magnesiumsulfat in die Blutbahn im Sinne einer Magnesiumsulfatnarkose erfolgt ist. Der in der Vorgeschichte geschilderte tragische Verlauf in unmittelbarem Anschluß an die Wurmkur kann als Beweis für die Richtigkeit dieser Annahme auf faßt werden."

Außer diesem Magnesium sulfuricum-Todesfall wurde, wie schon erwähnt, an anderer Stelle noch ein zweiter Todesfall nach peroraler Verabreichung von Bittersalz beobachtet und als dritter Todesfall nach einer Bandwurmkur der allerletzten Zeit wurde in der Medizinischen Klinik, 1952, 19, ein Filix mas-Todesfall von H. Greiner aus dem Institut für Gerichtliche Medizin an der Medizinischen Akademie Düsseldorf beschrieben, bei dem eine isolierte Herzwirkung im Vordergrund der Erscheinungen gestanden hat.

Es handelt sich also um 3 Todesfälle, die im Gefolge einer Bandwurmkur durch die Giftwirkung des verwendeten Abtreibemittels verursacht wurden. Vom wirksamen Prinzip des Filix mas, der amorphen Filixsäure = Filicin, ist seit langen Zeiten bekannt, daß es unter Umständen zu schwersten Komplikationen und sogar zum Tode führen kann, und zwar mitunter schon nach wenigen Stunden. An und für sich wird Filicin langsam und unvollständig resorbiert, wenn aber unter gewissen Umständen reichliche Mengen resorbiert werden, können verschiedengradige Vergiftungserscheinungen auftreten. Gleichzeitiger Alkoholgenuß fördert die Resorption, ist daher kontraindiziert. Auf die muskellähmende Wirkung des Filicins bei niederen Tieren dürfte der therapeutische Erfolg bei der Bandwurmkur zurückzuführen sein. Während es also seit langem bekannt ist, daß Filix mas-Präparate unter Umständen tödlich wirken können, ist es ganz neu, daß peroral zugeführtes Magnesium sulfuricum bei optimalen Resorptionsbedingungen auch den Tod herbeiführen kann.

Möller schreibt in seiner Pharmakologie, daß per os verabreichte Magnesiumsalze langsam und sehr unvollständig resorbiert werden, wodurch die therapeutische Anwendung dieser Stoffe als Abführmittel eine Erklärung findet. Wenn man aber Patienten mit mangelhafter Nierenfunktion wiederholt große Dosen per os gibt, so sieht man einen Anstieg der Magnesiumkonzentration im Plasma auf 10 bis 11 mg%, wodurch es zu einem komatösen Zustand kommt. Es wird auch behauptet, daß wiederholte Verabreichung abführender Dosen von Magnesiumsulfat bei Nephritispatienten ein Koma verursachen kann, das ganz dem urämischen Koma gleicht. Was verständlich ist, wenn man weiß, daß auch subkutan oder intramuskulär injizierte Magnesiumsalze schnell resorbiert und der größte Teil rasch, zur Hauptsache durch die Nieren, ausgeschieden wird. Daß bei parenteraler Magnesiumsalzzufuhr es auch beim Menschen zu einer vollständigen Narkose mit so tiefer Bewußtlosigkeit kommen kann, daß eine Operation durchgeführt werden konnte, ist auch bekannt. Man weiß auch, daß Magnesiumionen eine ausgesprochene Curarewirkung haben, so daß durch Reizung der Nerven keine Muskelkontraktionen ausgelöst werden können. Wegen dieser elektiven Wirkung war und ist das Magnesium das souveräne und unentbehrliche Mittel bei der Behandlung bzw. bei der Aufhebung der tetanischen Muskelsperre des Tetanus. Die therapeutische Wirkungsbreite für die tiefe Magnesiumnarkose ist aber sehr eng, weil die zur Auslösung einer Narkose notwendige Dosis bereits sehr nahe der atemlähmenden Dosis liegt, daher können neben der gewollten Muskellähmung auch schwere Störungen der Atmung auftreten. Es ist auch bekannt, daß die parenterale Zufuhr von Magnesiumsalzen bei einer Reihe von Versuchstieren bereits bei einer Plasma-Magnesium-Konzentration von [illegible]

* Für die Ueberlassung des Obduktionsbefundes und der zugehörigen Akten danke ich der Leitung des Gerichtlich-Medizinischen Institutes der Universität Graz (damaliger Leiter Doz. Dr. M. Fossel) bestens.

[illegible] Narkose vorgesucht; bei einer Konzentration von 15 [illegible] zu einer tiefen reflexlosen Narkose [illegible] zirka 20 mg% tritt seine Atemlähmung ein. In [illegible] Falle betrug der Magnesiumgehalt der frischen [illegible] pro 100 g [illegible] 24.5 und 25.5 mg%, also mehr als das Dreifache einer normalen frischen Lunge. Ein Befund, der wohl eindeutig für die Richtigkeit der Annahme spricht, daß in diesem Fall von dem innerlich verabreichten Magnesium ein sehr großer Teil resorbiert worden und dadurch zur Ursache der Narkose und der zentralen Atemlähmung geworden ist. Jetzt ist auch verständlich, da der Antagonismus Magnesium:Kalzium zugunsten des Magnesiums so groß war, daß das als Gegenmittel gespritzte Kalzium nur anfänglich imstande war, eine kurz dauernde Besserung herbeizuführen, später sich aber ganz wirkungslos erwies.

Zusammenfassend kann man sagen, daß sowohl Filix mas als auch Magnesium sulfuricum selbst bei **peroraler** Verabreichung so große Gefahrenmomente in sich bergen, daß man keinen Bandwurmträger diesen Gefahren aussetzen sollte. Der Rindenbandwurm, um den es sich ja in unseren Breiten hauptsächlich handelt, ist ja ein so harmloser Geselle, daß es unverantwortlich ist, seinetwegen den Bandwurmträger der Todesgefahr auszusetzen, besonders, nachdem wir in der Kalisiedersalzbandwurmkur ein so ungefährliches und gut bewährtes Mittel zur Hand haben. Wenn die erwähnten Kontraindikationen gegeben sind, so haben wir jederzeit die Möglichkeit, durch ein kräftiges Abführmittel einen Großteil des Bandwurmes zur Ausscheidung zu bringen, und der Bandwurmträger hat dann für zirka 2 bis 3 Monate, bis die Taenie wieder angewachsen ist, keinerlei Beeinträchtigung zu gewärtigen. Auf den wahrscheinlichen Einwurf, daß Filix mas doch äußerst selten zum tödlichen Ausgang führt, muß erwidert werden, daß dies nur dem Umstand zu danken ist, daß das Filicin nur langsam und unvollständig resorbiert wird. Wenn aber die Resorptionsbedingungen unter gewissen Umständen, die man nie voraussehen kann, äußerst günstige sind, wird die toxische und eventuell letale Dosis erreicht werden und der Fall eintreten, der angesichts anderer Möglichkeiten unbedingt vermieden werden sollte.

Literatur: Högler, F.: Ars medici, 1949, 12. — Klemperer: Neue dtsch. Klin. (Hetsch). — Kraus-Brugsch, Starkenstein, E. und Greiner, H.: Med. Klin., 1952, 19. — Meyer-Gottlieb: Pharmakologie. — Møller: Pharmakologie 1947, Handb. der experimentellen Pharmakologie, Erg.-Bd. X. — Schmidt, R.: Therapie und Prophylaxe innerer Krankheiten, 1948.

J. chron. Dis. 1969, Vol. 21, pp. 815–841. Pergamon Press. Printed in Great Britain

ESSENTIAL METALS IN MAN

MAGNESIUM*

HENRY A. SCHROEDER, M.D.,† ALEXIS P. NASON, B.S.
and
ISABEL H. TIPTON, Ph.D.

Department of Physiology, Dartmouth Medical School, Hanover, New Hampshire and the Brattleboro Memorial Hospital, Brattleboro, Vermont

(*Received* 1 *August* 1968; *in revised form* 2 *October* 1968)

MAGNESIUM, atomic number 12, is one of the four "bulk" metals in the human body, with calcium, potassium and sodium making up the major cations of the electrolytes. The "standard man", a 70 kg individual, contains 20 g magnesium, the largest part of it situated, like the 140 g potassium, intracellularly. There is more magnesium than calcium in most soft tissues and about 5 times more than the next most prevalent intracellular cation, zinc.

In terms of relative geochemical abundances, magnesium is the eighth most abundant element on the earth's crust, the sixth metal, the third divalent metal on land and the second in sea-water [1]. Therefore, large amounts of magnesium have been available for biological processes since the emergence of life on this planet. Animals depend on extracellular sodium and calcium and intracellular potassium and magnesium for transmembrane potentials, for osmotic equilibrium, for structure; many intracellular reactions are mediated by the latter two. In fact, some (if not most) insects use a magnesium-potassium system across cellular membranes for excitability and their hemolymph is rich in magnesium and poor in sodium and calcium [2].

Magnesium has been recognized as an essential nutrient since 1932, when KRUSE, ORENT and MCCOLLUM produced deficiency in the rat [3]. Subsequent studies on animals have demonstrated neuromuscular irritability, cardiovascular calcification and renal damage resulting from magnesium deficiency, which are prevented by supplementation. Magnesium, however, should be classed as a nutrient only in the sense that the other bulk metals, sodium, potassium and calcium are nutrients. As a major inorganic constituent of living things, and as a major intracellular cation, in the strictest sense it is not a nutrient.

This report is the seventeenth we have made on elements in human tissues and their sources in food, water and air in an attempt to evaluate whether or not deficiencies or excesses, if found, could be associated with chronic diseases. Magnesium

*Supported by grants in aid from the National Heart Institute, United States Public Health Service (HE 05076), U.S. Army Contract DA 2595, and CIBA Pharmaceutical Products Inc.
†Requests for reprints should be sent to 9 Belmont Avenue, Brattleboro, Vermont 05301.

is the only bulk element we have evaluated, because the others have been so well studied, because interest in magnesium is increasing and because suggestions have been made that the dietary intake of this element is marginal or deficient in the United States, and that cardiovascular disease could ensue [4, 5].

We asked ourselves several questions: What is the distribution of magnesium in various organs and tissues of the human body? Do concentrations change with aging or is homeostasis efficient throughout life? Do examples of tissue deficiency occur in human beings? If so, are they related to chronic diseases, especially cardiovascular? What foods supply considerable magnesium? Do modern industrial food practices contribute to less than adequate intakes?

Although there is much data in the literature on the magnesium content of foods, most analyses were made by older colorimetric methods and data on many foods are missing [4, 5]. Atomic absorption spectrophotometry is a more versatile, accurate method. Therefore, we have analyzed various common foods in an attempt to update and supplement values. We have discussed only briefly the extensive literature on the clinical, experimental and biochemical aspects of magnesium metabolism, as they have been well covered in recent reviews [4–8].

METHODS

The sources of the human tissues analyzed for magnesium have been described by TIPTON, *et al.* [9–11] and the method of analysis reported. In brief, tissues from mainland United States subjects were obtained from New York (New York), Baltimore (Maryland), Dallas (Texas), Memphis (Tennessee), Richmond (Virginia), Denver (Colorado), Miami (Florida), Tacoma and Seattle (Washington) and Chicago (Illinois). Tissues from newborn and young infants came from St. Louis (Missouri). In a subsequent study, tissues of subjects from San Francisco (California) were analyzed. The total number of subjects was 197. All of these samples were obtained frozen, ashed in muffle furnaces and analyzed by emission spectroscopy. The limit of detection for magnesium was 0.1 per cent ash (approximately 10 μg/g wet weight).

Foreign tissues were preserved in specially purified "metal free" formalin and ashed with the formalin. They were obtained from subjects in Honolulu, Anchorage (Alaska), Japan (three cities), Taiwan, Hong Kong, Manila, Bangkok, India (4 cities), Cairo, Beirut, Addis Ababa, Usumbura (Burundi), Kampala (Uganda), Welkom (Orange Free State), Lagos (Nigeria), and Lambarene (Gabon). Because of practical limitations of space for shipping, only 10 tissues from each cadaver were obtained. The total number of subjects was 202. Attempts were made to obtain tissues from African natives living in rural and primitive environments.

Statistical analyses of data were made as indicated by computer. In order to ascertain changes with age, the raw data were arranged according to decade of life and tabulated. Analyses were not done on all tissues of still-born and young infants and children below the age of ten years, of whom there were 46 United States and 2 foreign subjects. For some organs as few as 8 samples were analyzed; for liver and kidney 45 and 38 were recorded. In other decades of life there were the following numbers of subjects from the United States and foreign countries, respectively: Second, 11 and 15; third, 21 and 39; fourth, 42 and 51; fifth, 29 and 36; sixth, 30 and 22; seventh, 9 and 30; eighth and ninth, 9 and 7.

Foods and waters were analyzed by atomic absorption spectrophotometry (Perkin–

Elmer 303) using a Boling burner and a recorder readout. After ashing samples in muffle furnaces at 450°C., the ash was dissolved in 1 ml concentrated hydrochloric acid, diluted to 50 ml with deionized water and stored in polyethylene bottles. Aliquots were then diluted tenfold or more in order to use the working range of the apparatus. Limits of detection were not reached. The method was reproducible on ten consecutive analyses of the same sample at a level of 1 per cent. No sample was devoid of magnesium.

RESULTS

Concentrations and contents of magnesium in human organs

In Table 1 are shown the mean concentrations and the total amounts of magnesium

TABLE 1. MAGNESIUM IN HUMAN TISSUES, U.S. VALUES, 150 ACCIDENTAL DEATHS, MEAN VALUES

Organ	Organ weight (g)	Magnesium	
		Content (mg)	Concentration (μg/g wet wt ±S.E.M.)
Muscle	28,000	5300	189±7.2
Fat	12,500	300	24±0.2
Bone	10,000	11,000	1100±25.2
Blood	5500	210	38±0.4
Skin	4900	290	59±4.0
Connective tissue	2000	300*	150*
Liver	1800	310	172±5.2
Brain	1400	210	150±3.4
G.I. tract	1200	150	125±9.6
Lungs	1000	71	71±2.2
Heart	350	63	180±5.5
Kidneys	310	40	129±3.3
Spleen	180	23	128±4.2
Pancreas	100	16	160±4.4
Other	760	114*	150*
Total	70000	18397	285
Soft tissues	60000	9000	150

*Estimated from mean of all soft tissues. Analyses not done.
Note: Concentrations were obtained from analyses of 150 accidental deaths. Contents are based on organ weights of "standard man", using these concentrations. These 150 cases were selected from the total number analyzed on the basis of their dying accidentally and not of a known disease.

in major tissues and organs of the body of "standard man". Being an element in Group IIA of the Periodic Table, all elements of which are concentrated in bone, it was not surprising to find about 55 per cent of the total magnesium in the body in bone, at the highest concentration. Muscle, with the next highest concentration, contained 26.5 per cent of the total. The lowest concentration occurred in fat. There was more magnesium in muscle than calcium, and the mean concentration in soft tissues was about 150 per cent of that of calcium [12], although the total body content was only 2 per cent of that of calcium.

In Table 2 are shown the mean concentrations of magnesium in 20 other human organs and tissues. The lowest concentrations occurred in omentum and adrenal, which contain much fat, and the highest in larynx and aorta, which contain much

TABLE 2. MAGNESIUM IN OTHER HUMAN TISSUES, MEAN VALUES

	No. Samples	Ash (% of wet weight)	Magnesium g/100 g ash (±S.E.M.)	Magnesium μg/g wet weight (±S.E.M.)
Adrenal	10	0.5	0.89±0.10	45± 5.0
Aorta	94	1.7	1.5 ±0.06	255±10.2
Diaphragm	91	0.98	1.7 ±0.05	167± 4.9
Esophagus	57	0.89	1.4 ±0.06	125± 5.3
Stomach	110	0.82	1.5 ±0.04	123± 3.3
Duodenum	60	0.81	1.3 ±0.04	105± 3.2
Jejunum	83	0.92	1.3 ±0.06	120± 5.5
Ileum	77	0.75	2.1 ±0.12	158± 9.0
Cecum	31	0.67	3.1 ±0.25	208±16.8
Sigmoid colon	88	0.68	2.6 ±0.22	177±15.0
Rectum	41	0.85	2.6 ±0.19	221±16.1
Omentum	72	0.25	1.2 ±0.06	30± 1.5
Larynx	49	3.3	1.2 ±0.06	396±19.8
Trachea	57	1.9	1.2 ±0.05	228± 9.5
Thyroid	14	1.4	0.98±0.19	137±26.6
Prostate	40	1.2	1.6 ±0.06	192± 7.2
Testis	68	1.1	1.1 ±0.04	121± 4.4
Ovary	16	1.0	0.94±0.06	94± 5.9
Uterus	30	1.0	1.1 ±0.06	110± 6.0
Bladder	98	0.78	1.1 ±0.05	109± 3.9

calcium. The large intestine had more magnesium than did the upper gastrointestinal tract.

Association of magnesium with other elements

In Table 3 are shown paired rank coefficients of correlation of the concentrations of magnesium in 24 of 29 tissues and those of nine essential elements at a high level of significance ($p<0.001$). There were none at this level in adrenal, duodenum, larynx, ovary and skin. In the other 24 tissues, a rather remarkable association of magnesium with manganese appeared in 17, and with phosphorus in 14. Most of these tissues were from organs of high metabolic activity. An association with concentrations of copper occurred in 11, of zinc in 10 and of iron in 8. Magnesium concentrations were seldom associated with the two other bulk elements, or with cobalt. Therefore, this study failed to disclose many significant relationships of magnesium with calcium or with potassium.*

In Table 4 are shown similar coefficients of correlation of magnesium and 10 non-essential or "abnormal" trace elements in tissues. Most of them occurred in sections of the gastrointestinal tract. Barium was the most frequently associated element. One or more significant correlations at this level of significance were found in only 13 of the 29 tissues; coefficients were fairly high in sigmoid colon and rectum. They were not significant at this level for gold, boron, molybdenum or bismuth.

Change in tissue magnesium with age

In Figs. 1 and 2 are shown the mean concentrations of magnesium in 8 organs or

*By Chi-square analysis, magnesium was more frequently associated with phosphorus than with calcium ($p<0.025$) and with manganese than with iron ($p<0.05$).

TABLE 3. PAIRED RANK CORRELATION COEFFICEINTS OF MAGNESIUM AND ESSENTIAL ELEMENTS IN HUMAN TISSUES $p < 0{\cdot}001$

	K	P	Ca	Fe	Mn	Cu	Zn	Co	Cr
Brain	—	0.42	—	0.51	0.61	0.39	—	—	—
Heart	—	0.31	—	0.30	0.55	0.30	0.28	0.35	0.38
Aorta	—	0.53	—	—	—	—	—	—	—
Kidney	—	0.30	—	—	0.44	0.39	—	—	—
Liver	—	0.45	—	—	0.48	—	—	—	—
Lung	—	0.56	—	—	0.28	0.41	0.36	—	—
Pancreas	—	0.37	—	0.35	0.52	—	—	—	—
Spleen	—	0.47	—	0.51	0.37	0.34	0.30	—	0.29
Muscle	—	0.47	—	0.51	0.37	0.34	0.30	—	—
Diaphragm	—	0.43	—	0.36	0.41	—	0.38	—	—
Bladder	—	0.54	—	—	0.56	0.41	0.42	0.29	0.29
Uterus	—	—	—	—	0.53	0.55	—	—	—
Testis	—	—	—	—	0.69	—	0.35	—	—
Esophagus	—	0.44	—	—	0.38	—	—	—	0.57
Stomach	—	—	—	0.31	0.39	0.33	0.36	—	—
Jejunum	—	—	0.46	—	—	—	—	—	—
Ileum	0.47	—	0.59	—	—	—	—	—	—
Cecum	—	—	—	—	—	—	—	—	0.54
Sigmoid colon	—	—	0.61	—	0.51	0.39	—	—	—
Rectum	—	—	0.55	—	0.49	—	—	—	—
Omentum	—	0.57	—	—	0.59	0.44	0.50	—	—
Prostate	—	—	—	—	—	—	0.47	—	—
Thyroid	0.84	—	—	0.77	—	—	—	—	—
Trachea	—	0.46	—	—	—	—	—	—	—
Mean	0.655	0.451	0.553	0.453	0.481	0.390	0.372	0.320	0.414
No. of 29	2	14*	4	8	17*	11	10	2	5

*Differs by Chi-square analysis from K, $p < 0.005$.

TABLE 4. PAIRED RANK CORRELATION COEFFICIENTS OF MAGNESIUM AND "ABNORMAL" ELEMENTS IN TISSUES $p < 0.001$

	Ag	Al	Ba	Cd	Ni	Pb	Sn	Sr	Ti	V
Aorta	—	—	0.33	—	—	0.37	—	—	—	—
Brain	0.36	—	0.35	—	—	—	—	0.37	—	—
Heart	—	0.26	0.34	—	—	—	—	—	0.42	—
Pancreas	—	—	0.26	—	—	—	—	—	0.29	—
Diaphragm	—	—	—	—	—	0.32	—	—	0.32	—
Omentum	0.35	—	—	0.34	—	—	0.36	—	—	—
Stomach	—	0.32	0.28	—	—	—	—	—	—	—
Jejunum	—	0.33	0.32	—	—	—	—	—	—	—
Ileum	—	0.32	0.48	—	0.45	—	0.32	0.49	—	0.37
Sigmoid Colon	0.42	0.46	0.54	—	0.54	—	—	0.59	0.31	0.40
Rectum	—	0.51	0.60	—	0.44	—	0.45	0.41	—	—
Trachea	—	—	—	—	0·36	—	—	—	—	—
Bladder	—	—	—	—	—	—	0.32	—	—	—
Mean	0.377	0.367	0.389	0.340	0.448	0.345	0.363	0.465	0.335	0.385
No. of 29	3	6	9	1	4	2	4	4	4	2

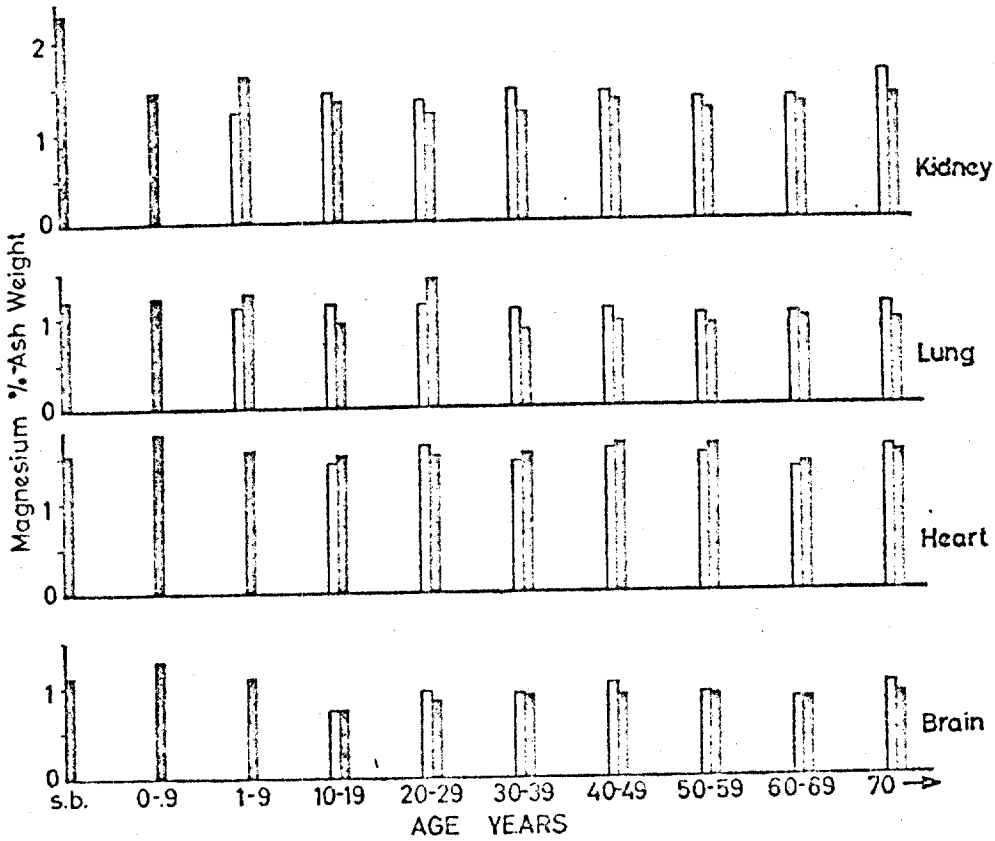


FIG. 1. Mean magnesium concentrations in ash of kidney, lung, heart and brain according to age. Black bars, U.S. tissues; open bars, foreign tissues. S.b. means still-born. In the first 3 columns, ages 0–9, there were 38 kidneys, 13 lungs, 12 hearts and 6 brains of U.S. subjects analyzed and only 2 foreign. The high mean value in still-born kidney is the result of two of 14 cases with aberrant levels and is not significant. For numbers in other groups, see text. Note the relative constancy of the values. There were 197 U.S. and 202 foreign subjects analyzed.

tissues of United States and foreign subjects according to decade of life. There were virtually no significant changes after the first decade. These values were calculated on the basis of ash. There were no significant age-linked correlations of these organs according to ash/wet weight ratios.

In comparing the mean concentrations of magnesium in the ash of the tissues of United States subjects in the first decade and last three decades of life, declines in the latter group were found for all but aorta, which rose 12.9 per cent. Older persons had the following percentages of tissue magnesium compared to growing children: Brain 75.9, heart 90.7, lung 74.6, kidney 70.2, liver 81.0, spleen 71.8, muscle 63.2, pancreas 81.3. Declines from the second to the seventh and eighth decades were found only in spleen (86.2 per cent).*

Like American tissues, magnesium concentrations in foreign tissues, where measured, did not usually decrease with age after the first decade of life. Levels in aorta,

*None of these age linked differences were statistically significant.

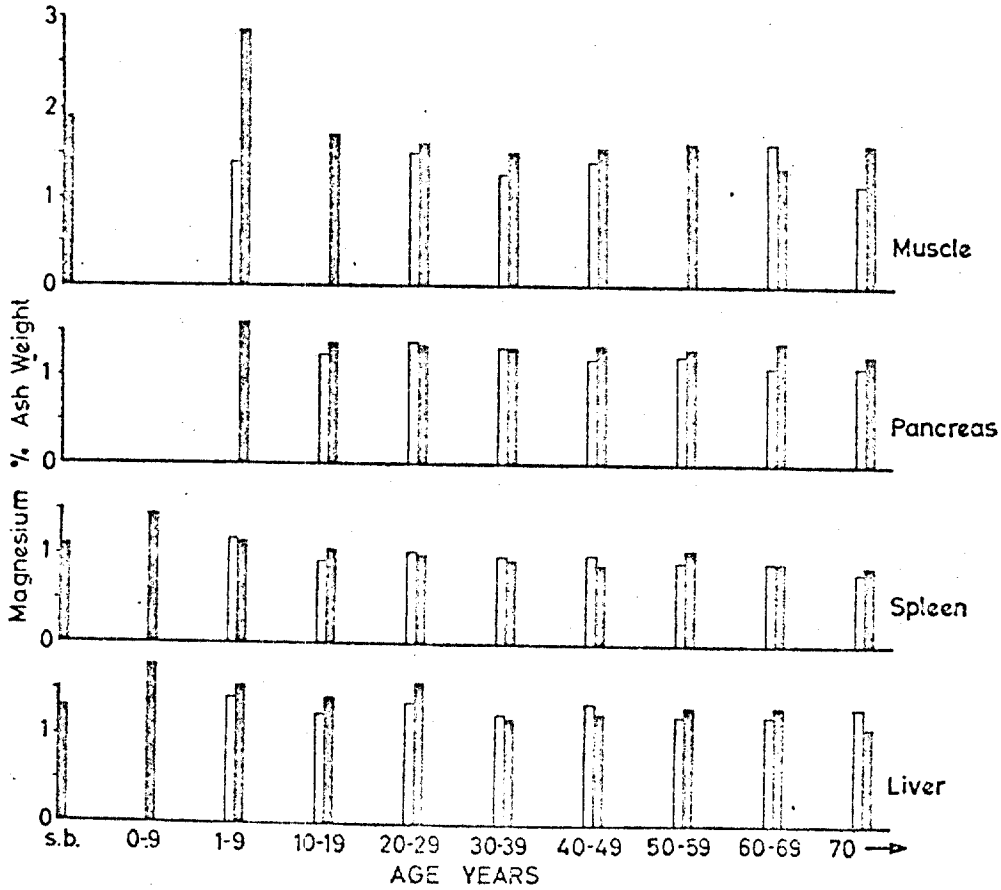


FIG. 2. Mean magnesium concentrations in ash of muscle, pancreas, spleen and liver according to age. Black bars, U.S. tissues; open bars, foreign tissues. S.b. means still-born. In the first 3 columns, ages 0–9, there were 45 livers, 9 muscles, 7 spleens and 2 pancreases of U.S. subjects analyzed, and only 2 foreign. The high value in muscle came from two cases with 3.7 and 2.0 μg/g. There were only 2 cases in the age group 0–0.9 yr.

brain, heart, lung, liver and kidney were quite constant in all decades, except for a slight rise in the last. Mean levels in spleen and pancreas fell slightly (82.7 and 89.4 per cent respectively) from the second to the seventh and eighth decades.

Figure 3 shows the concentration of magnesium in the aortas of United States subjects according to age on the basis of wet weight, compared to the amount of ash in the aorta. The curves are parallel, and no significant change in magnesium in aortic ash occurred. Superimposed on these curves is that for calcium, which also increased three-fold with age. It is apparent that the changes due to aging, presumed to be associated with atherosclerosis, are accompanied by profound alterations in the concentrations of these two metals in the aorta. In ash there was a decline in copper ($r=-0.51$) and potassium ($r=-0.70$) with age ($p<0.001$), but concentrations in wet tissue remained relatively constant throughout life, indicating that changes were related to the amounts of calcium and magnesium and their salts.

The mean values of all aortas in this series showed that 1.7 per cent of the wet

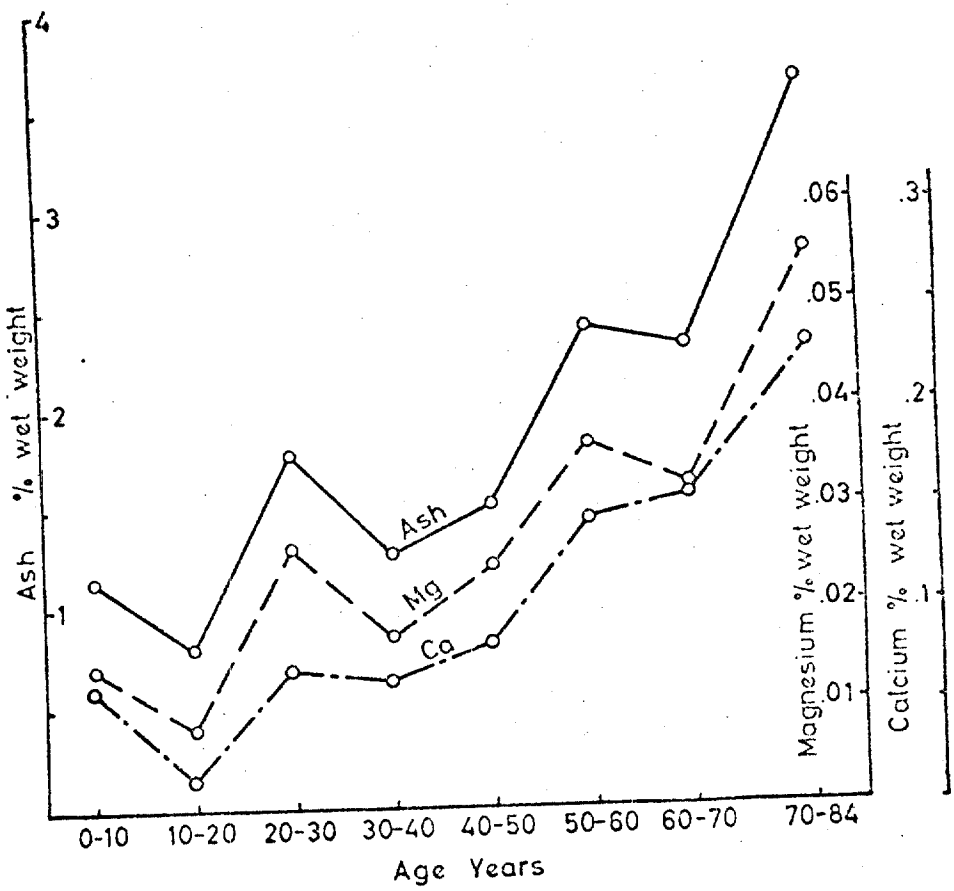


FIG. 3. Mean concentrations in aorta, according to age, of ash, magnesium and calcium in per cent of wet weight, U.S. subjects. Note the progressive rise beginning in the fifth decade of life, presumably with the advance of atherosclerotic changes. Calcium and magnesium levels according to ash weight were relatively constant. There were 139 aortas analyzed. Potassium and copper concentrations declined significantly with age according to ash but were relatively constant according to wet weight. Paired rank correlation coefficients with age were: wet weight, calcium +0.91, magnesium +0.77; ash weight, potassium —0.70, copper —0.51, calcium +0.43; ash per cent of wet weight, +0.45; dry per cent of wet weight, —0.39; ash per cent of dry weight +0.51 ($p < 0.001$). Wet weights were not available for foreign aortas; magnesium in ash was quite constant, varying between 1.34 and 1.47 per cent from the second to the seventh decades, and rising to 2.0 per cent in the eighth.

weight and 5.1 per cent of the dry weight was ash. Of this ash, there was (±S.E.M.) 5.4±0.24 per cent calcium, 1.5±0.06 per cent magnesium, 6.0±0.20 per cent phosphorus, 0.33±0.02 per cent iron, 9.0±0.08 per cent potassium and 0.19±0.07 per cent zinc, making up 22.42 per cent of the ash weight. The atomic ratio of magnesium to calcium in the aorta of the dog was approximately 1:1 (13); in the human aortas of all ages it was 0.46, in young aortas (<20 yr of age), 0.83 and in older aortas (>50 yr of age) 0.39. The ratio declined progressively, beginning in the third decade of life ($r = -0.90$, $p < 0.005$).

Low and high values of magnesium

Because magnesium is mainly intracellular, except for that portion bound in bone, wasting disease would theoretically release magnesium from cells, whereas it would be conserved in healthy people. If renal homeostatic mechanisms for magnesium are altered by disease, subjects with uremia and hypermagnesemia might be expected to show high tissue concentrations, unless active celluar transport mechanisms prevented excess penetration of the cellular membrane.

At a level of twice the mean for a decade, there were no excessively high concentrations of magnesium found in any human tissues, even in 7 subjects dying of uremia or renal insufficiency (Table 5), except possibly in brain. Concentrations one half

TABLE 5. MAGNESIUM IN TISSUES OF SUBJECTS DYING OF CHRONIC RENAL INSUFFICIENCY AND ALCOHOLISM (g/100 g ASH±S.D.)

	Control	Renal failure	Toxemia of pregnancy	Alcoholism
No. cases	150	7	2	5
Brain	0.96±0.25	1.52±0.73*	1.0, 1.0	0.97±0.17
Heart	1.60±0.51	1.69±0.45	2.0, 1.1	2.04±0.76
Lungs	1.02±0.25	1.05±0.26	0.98, 1.9	1.08±0.25
Liver	1.28±0.41	1.38±0.33	1.2, 0.88	1.38±0.28
Kidney	1.35±0.35	1.28±0.39	1.6, 1.4	1.69±0.55
Spleen	0.94±0.31	0.91±0.26	0.6, 0.8	1.22±0.44
Pancreas	1.30±0.43	1.20±0.57	1.5	1.64±0.50
Muscle	1.60±0.61	1.65±0.46	—	2.08±0.84

*Two subjects had 1.9 and 3.0 μg/100 g magnesium, both values exceeding extreme ranges for non-uremic subjects.

Note: Four of the subjects with renal failure or uremia had malignant hypertension; 3 had chronic renal disease. They were all foreign cases. The five patients with chronic alcoholism were United States subjects.

of the mean or less for 1 or more organs by decade were found in seven United States and 5 foreign subjects. All deaths in the domestic group were from accidents at ages 20–69 yr; two foreign deaths were of unknown causes; one was from pulmonary tuberculosis, one from hypertensive disease and one from coronary occlusion with heart failure. Wasting diseases such as chronic infections and cancer were not usually accompanied by low tissue magnesium. There were only two examples of single values of one-third of the mean in a decade's samples, both in brain. Five subjects dying of chronic alcoholism had normal concentrations of magnesium in soft tissues (Table 5).

Sources of magnesium: Foods

Concentrations of magnesium in some common foods are shown in Table 6. As is true for the essential trace metals we have examined, spices contained the most. Nuts and whole grains were excellent sources. Fats were almost devoid of magnesium. The concentrations in meats and fish were higher than that in human muscle. The values found in foods were generally similar to those obtained by older methods [14], except for fats and some sea foods.

In order of decreasing mean concentrations of magnesium by weight, foods could be ranked as follows: Nuts, cereals, sea foods, meats, legumes, vegetables, dairy

TABLE 6. MAGNESIUM IN FOODS (WET WEIGHT)

	μg/g		mg/100 kcal
Fish and sea food			
Blue Gill, raw, frozen	375		31.7
Cod, raw, frozen (3)	249	(207–285)	30.4
Haddock, raw, frozen (2)	484	(436–532)	68.2
Haddock, cooked, chowder (2)	359	(328–389)	37.0
King Crab, cooked, frozen	530		41.7
Lobster, cooked, frozen	376		31.6
Oyster, raw, frozen	154		30.8
Scallops, cooked, frozen (6)	335		31.9
Smelt, raw, frozen	237		28.2
Tuna, canned	334		34.4
Mean	348		36.6
Meat			
Beef and fat	402		14.7
Beef Liver	203		14.2
Chicken leg	255		12.6
Chicken wing	195		9.6
Ground round steak	258		8.9
Lamb chop (2)	249	(239–258)	13.3
Lamb liver	234		16.4
Pork loin	209		7.4
Top round steak	383		15.4
Gelatin, unflavored	283		35.8
Mean	267		14.8
Dairy products			
Cheese, cheddar (2)	268	(266–270)	6.3
Egg, whole	113		6.9
Egg, white	103		27.8
Egg, yolk (2)	101	(100–101)	2.9
Milk, whole, homog. vit. D	102		15.5
Milk, skim, powdered	1067		32.7
Milk, human (2)	29	(28–29)	3.8
Milk, fat free	162		46.3
Milk "cereal cream" 12% butter fat plus milk solids	280		6.9
Mean, less dry milk	157		18.2
Vegetables, fresh			
Leafy			
Broccoli	321		229.3
Brussels sprouts	233		145.6
Cabbage	166		63.8
Chicory	94		104.4
Chives	241		219.1
Endive	160		145.5
Escarole	89		80.9
Lettuce, green, outer	139		126.4
Lettuce, pale, inner	85		77.3
Parsley	566		269.5
Mean, less parsley	170		146.2

Table 6. (*continued*)

	μg/g		mg/100 kcal
Vegetables, fresh			
Roots			
Carrots	185	(111–296)	80.4
Leek	264		105.6
Leek greens	478		191.2
Onion (2)	89	(75–103)	38.7
Parsnips	456		93.1
Potatoes (2)	167	(155–179)	19.2
Radish	162		108.0
Scallions	137		38.1
Turnip	95		52.8
Mean	226		80.8
Vegetables, fresh			
Legumes			
Beans, green, string	297		92.8
Beans, wax, yellow	241		89.3
Beans, Lima, dry (2)	1697	(1445–1948)	49.2
Beans, kidney, dry	775		22.4
Pencil pod, black, dry	1713		50.4
Peas, green	185		22.0
Pea, green, dry	1182		34.0
Mean, fresh	241		51.4
Vegetables, fresh			
Fleshy			
Artichoke, globe	487		324.7
Cauliflower	229		208.2
Celery	66		73.3
Cucumber	107		118.9
Egg plant	82		54.7
Mushroom	122		174.3
Pepper, green (3)	102	(92–114)	46.4
Squash, butternut	165		30.5
Squash, yellow	137		72.1
Squash, zuccini	237		139.4
Tomato (3)	177	(121–241)	126.4
Mean	174		124.4
Fruits and fruit juices			
Apple	58		12.9
Pear	64		16.0
Grape juice, canned	80		12.1
Orange juice	108		28.4
Papaya	0.1		< 0.1
Mean, less papaya	78		17.4
Nuts			
Brazil nut	3175		49.3
Walnut	1315		24.0
Hazel nut	1739		29.0
Almond	2927		48.9
Peanut	1583		26.3
Pecan	1078		18.0
Mean	1970		32.6

Table 6. (*continued*)

	μg/g	mg/100 kcal
Grains and cereal products		
Barley	1505	41.8
Buckwheat	2526	70.2
Corn, hybrid	664	69.2
Corn, late sunshine	394	41.0
Corn, meal, yellow	269	7.6
Corn starch	22	0.6
Cream filled doughnut	185	4.6
Kellog's Special K	412	12.2
Kellogg's concentrate	574	15.6
Millet	1670	47.7
Egg noodles	702	19.9
Oats	1705	42.2
Puffed rice	150	4.3
Pumpernickel	550	22.0
Wheat, flour, patent	299	8.5
Wheat, gluten	18	0.5
Wheat bread	204	7.7
Whole wheat bread	340	13.8
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
Mean	[illegible]	[illegible]
Oils and fats		
Butter, lightly salted (2)	24 (20-27)	[illegible]
Butter, unsalted	6	< 0.1
Cod liver oil	1	
Cottonseed oil	1	< 0.1
Peanut oil	8	0.1
Pork fat	5	< 0.1
Sunflower oil	6	< 0.1
Tallow, beef (2)	5 (2-8)	< 0.1
Mean	7	< 0.1
Condiments, spices, etc.		
Allspice, ground	1783	71.3
Cloves, whole	2549	101.9
Chili powder	3329	97.9
Garlic, fresh	230	16.8
Iodized salt	1667	—
Mustard, ground	4225	91.3
Pepper, black, ground	2323	75.2
Thyme, ground	615	24.6
Mean	2598	71.7
Beverages		
Beer	100	23.8
Cocoa, powdered	4289	94.9
Coffee, 97% caffein free, dry powder	6362 (5624–7143)	—
Coffee, infusion	48	96.0
Gin	0.3	< 0.1
Grape wine, white	98	11.5

Table 6. (*continued*)

	μg/g		mg/100 kcal
Tea, black, infusion	11		110.0
Tea, black, leaves	2183		—
Tea, Japanaese green, infusion	3		30.0
Tea, Japanese green, leaves	1666		—
Vermouth, Italian	135		9.9
Whisky, bourbon	1.3		<0.1
Whisky, scotch	4.5		0.2
Sugars and syrups			
Corn syrup (3)	17	(14–23)	0.6
Honey	15		0.5
Jelly, mild cider	215		8.3
Jelly, wild cherry	32		1.2
Jelly, thyme-grape	33		1.3
Maple syrup, Vermont fancy (2)	150	(136–164)	5.1
Molasses, beet	110		4.3
Molasses, cane (4)	2093	(1311–2526)	81.4
Raw sugar, Columbian	122		3.1
Raw sugar, Phillipine	66		1.7
Sugar cane, green	190		—
Sugar crystals, cane, England	3		<0.1
Sugar, white, crystals, U.S.	3		<0.1
Sugar, white, France	2		<0.1
Sugar, white, granulated, U.S.	2		<0.1
Mean, less cane and molasses	59		2.0
Animal feeds and feed additives			
Alfalfa	1208		
Cat diet, chow	1716		
Cattle feed, pellets	2302		
Cattle feed, powder	3563		
Hay, partially dried	398		
Lab diet, chow	1968		
Protein hydrolysates (2)	419	(338–500)	
Rat food 1966	820		
Rat food 1967	957		
Torula yeast rat diet 1965	1879		
Torula yeast rat diet 1967	915		
Fish flour rat diet	1525		
Mean	1473		
Miscellaneous			
Brain, deer	113		
Praying mantis, green	979		
Praying mantis, brown	1230		
Cecropia Moth, tissues	678		
Cecropia Moth, hemolymph	2423		
Cecropia Moth, pupa, whole	746		
Washed human blood cells (2)	53	(49–57)	
Hair, human, male (47)	49.7±8.63		
Hair, human, female (30)	85.7±13.83		
Mouse, liver (9)	233		
Mouse, muscle (9)	285		
Mouse, kidney (9)	188		
Seaweed, Caribbean	13,658		
20% superphosphate	8,876		

Numbers in parentheses indicate numbers of samples analyzed.

products, fruits, refined sugars, fats. Because of low caloric values of many vegetables, this order differed when concentrations were ranked on the basis of caloric values of the foods, as follows: Vegetables, legumes, sea food, nuts, cereals, dairy products, fruits, meat, refined sugars, fats. The very small contribution of fats and refined sugars to the total intake of magnesium is noteworthy.

It is generally believed that green leafy vegetables are a major source of magnesium, owing to the presence of magnesium in the porphyrin complex of chlorophyll.* While this belief is true on a caloric basis, it is not on a weight basis. The nine green leafy vegetables in Table 6 had a mean concentration of magnesium of 170 μg/g, the eight root vegetables 194 μg/g, the three legumes 241 μg/g and the 11 vinous or fleshy vegetables 174 μg/g. On the other hand, where analyses were made on the white or green parts of the same plant, as was the case for lettuce and leaks, the white portion contained 55–61 per cent as much magnesium as the green.

The low values in wheat gluten suggest that the magnesium in the endosperm of wheat is associated mainly with the starch and not the protein. This distribution does not hold for corn, starch being low in magnesium.

The table also shows the concentrations of magnesium in some animal foods, which are generally considerably higher than in human foods. Values rank with some whole grains. The high concentrations in the whole bodies of three insects is interesting in the light of the high concentrations of magnesium in hemolymph and low concentrations of sodium [2] in moths, the only known example of a divalent cation being used as a major constituent of extracellular fluids. The concentrations of magnesium in these insect tissues was 4 to 6 times that of human soft tissues, 2 to 3 times that of the whole human body and in the range of that in human bone.

The values in seaweed equal those of seawater. The values in superphosphate fertilizer are such as to supply adequate amounts to growing foods.

Losses of magnesium apparently caused by refining some raw foods or by cooking are shown in Table 7. The refining of whole wheat to fine patent flour resulted in a loss of 80 per cent of the magnesium, as it did for the essential trace elements [12, 15, 16]. Consequently the less refined but edible portions of the grain were enriched two-fold or more. The polishing of rice may remove more than 80 per cent of the magnesium. The refinement of sugar removed nearly all of the magnesium in sugar cane or in raw sugar, with a consequent marked enrichment of molasses.† Boiling vegetables in water removed more than half of the magnesium from celery, two thirds from carrots and nearly three quarters from parsnips.

The amounts of magnesium in the standard diets we have used for analysis are given in Table 8. For a 70 kg individual they would provide approximately 3 mg/kg body weight per day, a value considerably below that found experimentally to produce magnesium balance between intake and output [4].

Sources of magnesium: Water

Hard water contains the salts of magnesium and calcium; soft water has little of

*Chlorophyll contains 2.72 per cent magnesium by weight; it makes up 8 per cent of chloroplasts. 100 g chloroplasts would contain 217.6 mg of magnesium and produce about 400 kcal of energy, or 54.4 mg magnesium per 100 kcal.

†It is interesting, from a nutritional viewpoint, that the enriched residues from the refined carbohydrates of wheat and sugar cane are fed to poultry and livestock, whereas human beings eat the depleted material.

TABLE 7. EXAMPLES OF DEPLETION OF MAGNESIUM IN FOODS BY REFINING AND COOKING

	μg/g	Percentage of raw product (%)
Wheat flour, grades		
Patent	299	19.9
First clear	658	43.8
Second clear (low grade)	1297	86.4
Whole wheat	1502	—
Red dog	3217	214.2
Shorts	5212	347.0
Germ	3649	242.9
Bran	5979	398.1
Gluten	18	1.2
Rice		
Unpolished	1477	—
Polished	251	17.0
Corn		
Whole	664	—
Meal	269	40.5
Starch	22	3.3
Sugar		
Sugar cane	190	—
Raw Columbian	122 } 94	—
Raw Phillipine	66 }	—
Refined, white	2	2.1
Vegetables		
Carrots, raw	185	—
Carrots, cooked	62	33.5
Carrots, cooking water	119	—
Celery, raw	66	—
Celery, cooked	31	47.0
Celery, cooking water	119	—
Parsnips, raw	456	—
Parsnips, cooked	126	27.6

TABLE 8. MAGNESIUM IN HOSPITAL AND INSTITUTIONAL DIETS

	Weight (g)	Percentage of dry substance	μg/g	Total (mg)
Hospital				
Breakfast	518	18.6	124	64.2
Dinner	519	21.3	174	90.3
Supper	660	21.6	66	43.6
Total	1697	20.5	117	198.1
Institutional				
Breakfast	766	25.8	116	88.9
Dinner	861	18.4	93	80.1
Supper (estimated)	899	19.1	60	54.0
Total	2526	21.1	88	222.0

Note: The composition of the diets have been reported [12].
Supply of institution supper was depleted, value estimated from standard tables [14].

these elements. The degree of hardness of water depends largely on soluble calcium and magnesium. The median hardness of finished municipal water supplies of the 100 largest cities of the United States is 90 ppm, with 26 ppm calcium and 6.25 ppm magnesium, a ratio of about 4:1 [17]. Maximal values were 738, 145 and 120 ppm, respectively, with a Ca:Mg ratio of about 1.2:1. No calcium or magnesium were detected in the softest waters. If a person drank 2 l. local tap water per day*, he would consume 12.5 magnesium at median water hardness, 240 mg at maximal and none at minimal hardness. In cities, 23 million people drink water of less than 61 ppm hardness (magnesium less than 5 ppm), 22 million, water of 61–121 ppm (magnesium 5–8 ppm), 11 million, water of 121–180 ppm (magnesium 8–15 ppm) and 3.7 million, water of more than 180 ppm (magnesium>15 ppm) [17]. Therefore, most city dwellers receive only a small part of their daily magnesium intake from water.

In Table 9 are shown magnesium in a few waters. A water in Ohio would provide 33 mg magnesium and one in New Hampshire 28 mg daily. These waters would account for an additional 15 per cent of the magnesium in foods containing 200 mg per day.

TABLE 9. MAGNESIUM IN SOME WATERS

	ppm
Natural waters	
Sea water, Caribbean	1300
Lake George, N.Y.	2.0
Lake Spofford, N.H.	8.4
Connecticut, River, Brattleboro, Vt.	23.0
Springs	
Brattleboro, Vt.	2.4
West Brattleboro, Vt.	0.8
Silverdale, N.H.	14.1
Chesterfield, N.H.	3.6
Saratoga, N.Y.(State Seal water)	5.3
Well, Putney, Vt.	5.2
Municipal waters	
Bangor, Penn. (Roseto)	0.8
Brattleboro, Vt.	1.4
Bridgeport, Conn.	3.8
Gibsonville, Ohio	16.4
Peterborough, N.H.	1.3
White Pains, N.Y	1.7

DISCUSSION

We can now examine magnesium in the light of its abundance relative to other bulk inorganic components of the body and its role in human metabolism as a major intracellular electrolyte. In the "standard man" there are the following amounts of bulk elements (g): calcium 1000; phosphorus 840; sulfur 160; potassium 140; sodium 110; chlorine 100; magnesium 20; silicon 18. After them in abundance are the trace elements iron 4.9 g, zinc 2.4 g and fluorine 2.6 g, with the remainder in milligram quantities.

Because no appreciable declines in mean magnesium concentrations were found with age in any tissue analyzed, it is reasonable to assume that magnesium homeo-

*The national sales of soft drinks has been estimated at about 9 bottles or cans per person per day, or 2.4 to 3 l. The water used in the manufacture of soft drinks is softened to a standard value. How much tap water is actually drunk is unknown.

statis was operative throughout life in the subjects examined. The question of marginal dietary intakes and negative magnesium balances has been raised [4, 5, 18 and 19], however, and must be seriously considered, for there is a dichotomy between the results of nutritional balance studies and metabolic studies which awaits a sensible solution.

From the viewpoint of the evolutionary dietary history of the human race, it is logical to believe that every major natural source of caloric energy carries with it the inorganic nutrients necessary for its metabolism. This statement holds for all animals; otherwise deficiencies would occur and species would either become extinct or would have to depend on more than one source of food for survival (i.e. a "balanced" diet).*

1. *Is the dietary intake of magnesium marginal?*

Magnesium is required for synthesis and breakdown of glycogen, for breakdown of fatty acids, for glucose metabolism, and for many, if not all, reactions involving phosphorylation [20]. Considering magnesium as an inorganic nutrient only in the sense that it is a cofactor for many enzymatic reactions, this element should be expected (and is present) in all raw carbohydrate and protein foods and those which contain fat.

When raw foods are separated as to their major constituents, one or another fraction may contain levels of micronutrients suboptimal for adequate metabolism [12, 15, 21, 22]. Our analyses indicate that similar results of this separation apply to magnesium in several instances: milk, fat-free milk and butter; beef and tallow; pork and pork fat; seeds and their oils; corn, corn meal and corn starch; peanuts and peanut oil; whole wheat and its fractions, including gluten; whole rice and polished rice (Tables 6 and 7). In all of these cases the last mentioned fraction of raw food contained less, little, or negligible quantities of one of the elements presumed necessary for its metabolism, magnesium. Similar separations have been observed in the cases of zinc, copper, manganese, chromium and iron in fractions of refined foods [12, 15, 16, 21–23].

On the basis of our analyses and from indirect evidence [4, 19], a good argument for the existence of dietary magnesium definciency in this country can be made. Two of the major sources of caloric energy, refined sugars and fats, are virtually devoid of magnesium.† A third major source, wheat, is refined so as to lose 80 per cent, and other carbohydrate grains, rice and corn, are often polished or treated to remove most of their magnesium. The wide-spread practice of boiling vegetables can remove magnesium.

Under these conditions, and assuming SEELIG's requirement for magnesium of 6 mg per kg body weight per day [4] (420 mg for a 70 kg man), it is clear that dietary

*Balanced diets are necessary for domestic and laboratory animals and for man, because pastures are overcropped or otherwise depleted of trace elements, foods are prepared from many sources, and are processed in a variety of ways. We know of no wild animal, terrestrial or marine, requiring a "balanced" diet.

†BORST [24] advocated a high fat, high carbohydrate, protein-free diet (butter-sugar diet) for the symptomatic treatment of uremia, with good temporary results. It is possible that one of the virtues of this diet, which one of us (HAS) has used, is the result of the negligible intake of magnesium, which can contribute to uremic symptoms when renal mechanisms for the excretion of magnesium have deteriorated [7].

deficiency should be expected and that it should be quite prevalent in the United States. In fact, any diet containing less than 300 mg of magnesium (4.3 mg per kg), or 210 mg (3.0 mg per kg), which caused negative balances [19], of which there must be many, might be considered deficient for optimal nutritive requirements and serious chronic diseases could theoretically result from their use. Atherosclerosis has been often suspected of being the result of dietary magnesium deficiency [4, 5, 18, 25–27].

From our data, diets low and high in magnesium can be made, using means in Table 6 for various types of foods. A low magnesium (and probably atherogenic) diet of 2500 kcal would result if 45 per cent of calories came from fat (traces of magnesium) 22 per cent from sugar (traces of magnesium), 10 per cent from dairy products, 10 per cent from white flour, 10 per cent from meat and 3 per cent from vegetables and fruits, containing 120 mg magnesium. By substituting seafood, whole grain products, nuts and vegetables for half the fat and half the sugar, a diet containing 630 mg can readily be constructed. It is clear from the values in Tables 6 that the major sources of energy, refined flour, refined sugar and fat are poor in magnesium; if 90 per cent of the energy in a 3500 Cal diet came equally from these sources, it would contain only 110 mg magnesium.

In spite of the probability of diets being low in magnesium under certain circumstances, magnesium deficiency does not occur in human beings with healthy kidneys [7]. BARNES, COPE and HARRISON [28] were the first to offer an explanation. By feeding four healthy females a diet virtually devoid of magnesium for 18 days, they showed that renal mechanisms were efficient enough to conserve all but about 1 mEq magnesium per day (12 mg). Fecal losses were minor. Therefore renal homeostatic mechanisms for conserving magnesium are very efficient, as they are for sodium; they are probably more efficient than those for potassium or calcium.* Theoretically, then, extracellular magnesium deficiency would occur with low intakes in the presence of magnesium-losing nephritis, or by excessive losses from sweat and feces, or in extreme degrees of malabsorption. Theoretically also, cellular magnesium deficiency should be no more prevalent than potassium deficiency, occurring only in severe disorders.

2. *Basic reactions*

Magnesium has a single divalent state, and does not form highly stable chelates with organic complexes, as do the transitional metals. Perhaps this quality allows it to act as a bridge in a large number of chemical reactions not requiring redox reactions, but resulting in transfers of organic groups from one molecule to another. Examination of the classes of enzymes listed by DIXON and WEBB [20] show that when organic phosphate takes part in a reaction, magnesium is usually the inorganic cofactor. Although the list of metal-activated and specific metalloenzymes is incomplete, magnesium is a required cofactor in one oxidoreductase, 63 transferases, seven phosphoric monoester hydrolases, five lyases, and 20 ligases; phosphate is

*LOWE [53] fed 10 patients with peptic ulcers a synthetic diet free of electrolytes for 14 days. Mean lowest urinary excretions per day were as follows (mEq): Na, 8.8; K, 9.0; Mg<1.0. Whereas his data indicate better conservation of magnesium on a weight basis, they suggest different degrees of conservation in relation to filtered load, indicating that 0.03 per cent of the sodium, 0.05 per cent of the chloride, 1.18 per cent of the potassium, and 0.3 per cent of the magnesium in glomerular filtrate was excreted in the urine under conditions of total deprivation. We do not know the obligatory renal loss of calcium.

required in all of these reactions. On the other hand, magnesium apparently takes part in no mammalian reactions where phosphate is not involved, although a few bacteria use magnesium without phosphate. The association of magnesium with phosphate occurs in many tissues (Table 3).

Chelates of magnesium are usually quite readily dissociable [29]. Logs of the stability constants of magnesium and various phosphate complexes are low: phosphate 2.87; adenosine triphosphate, 3.61; adenosine diphosphate, 3.04; adenosine monophosphate 2.00; guanosine triphosphate, 4.02; pyrophosphate, 5.70; triphosphate, 5.81 (cf. EDTA 9.72). Perhaps this quality of dissociability allows magnesium to take part in so many reactions involving organic phosphates.

3. *Absorption and excretion*

Ingested magnesium is absorbed mainly by the small intestine, the proportion being dependant upon the dietary intake [7]. Because calcium and magnesium probably have common absorptive sites, the former divalent metal may compete with the latter, and partly inhibit absorption of magnesium when calcium is present in large amounts. Magnesium is also absorbed by the colon. Some mechanism for controlling excessive absorption must be present in the intestinal tract; GRAHAM *et al.* [30] found that at dietary intakes of 1.9, 20 and 47 mEq per day, 75.8, 44.3, and 23.7 per cent of ^{28}Mg given orally were absorbed, respectively, or 1.44, 8.86 and 11.14 mEq of stable isotope.

There is undoubtedly considerable excretion of magnesium into the intestinal tract, contained in bile, pancreatic and intestinal juices, with consequent reabsorption, but the amount not reabsorbed is small (1–2 per cent of ^{28}Mg given intravenously appeared in feces [31]. It is likely that the amount in different secretions varies considerably. Saliva contains 5–10 mg per l. and pancreatic juice only 1.7 mg per l. [32]. In patients suffering from large losses of intestinal fluids, hypomagnesemia can occur, suggesting that intestinal juices contain enough magnesium to deplete serum when not reabsorbed by the colon.

Serum levels are remarkably constant in healthy people, 1.7 mEq per l. [33], varying less than 15 per cent from the mean, "indicating that the serum concentration is maintained by sensitive control mechanisms that are poorly understood at present", according to WACKER and PARISI [7]. The principal mechanism is in the kidney. The obligatory urinary loss appears to be about 12 mg per day* [28]; the maximal renal capacity for excretion is not known, but is quite high†, perhaps more than 2 g per day [7].

According to SILVER *et al.* [31], the turnover rate of injected radioactive magnesium

*It is likely that the obligatory loss of magnesium is much less than 1 mEq per day. On a diet containing 8 mg of magnesium [54], SHILS found, by atomic absorption spectrophotometry, 0.05–0.09 mEq per day in 2 subjects (mean 0·08 mEq) after 24 and 92 days of depletion, respectively, 0.07 mEq in 1 subject after 100 days, and 0.14 mEq after 50 days depletion in a fourth. Analyses by the Titan Yellow method gave results 3–6 times higher, but in all of 4 subjects, excretion was less than 1 mEq per day (SHILS, M.E. personal communication). It is probable that virtually all (< 1 mg) magnesium is reabsorbed by the human renal tubules under conditions of severe deficiency if the atomic absorption method is accurate.

†CHESLEY and TEPPER [55] infused magnesium salts into normal subjects and patients with renal disease. The clearance of magnesium increased linearly with the serum level, and the ratio of the clearances of magnesium to inulin approached 1 at the highest serum levels. Therefore, the renal excretory limit of magnesium appeared to be the inulin clearance.

in the body is slow, less than 2 per cent for 25–50 per cent of the body's total magnesium. There was a slow component with a half-time of 14–35 hr for 10–15 per cent and two fast components of 1 and 3 hr for 15–25 per cent of the injected dose. It is likely that some bone stores are exchangeable.

Amounts of magnesium absorbed are excreted by the kidney; fecal magnesium represents largely the unabsorbed fraction [7]. Magnesium filtered by the glomeruli is reabsorbed by the renal tubules, probably actively, although the control mechanisms are not known. FARNSWORTH and KRAKUSIN [34] were the first to show that mercurial diuretics caused excretion of magnesium as well as potassium and calcium, as was shown many years before for sodium [35]. These observations suggest that mercurial diuretics act on tubular reabsorptive sites common to all four bulk cations. Thiazide diuretics produce the same cation losses.

Part of the magnesium in plasma is in the ionic form, the remainder being bound to protein. About 65 per cent of the total, or less, depending upon total concentration, is ionic, and it is this fraction which largely appears in glomerular filtrate. Any filtered protein-bound magnesium probably normally returns to the circulation via lymph; in renal diseases with heavy proteinuria magnesium may be excreted in larger than normal amounts.

Excretion also occurs in sweat. CONSOLAZIO *et al.* [36] exposed men for 7.5 hr to a 37° C temperature for 16 days on a balance study. From 10.1 to 15.4 per cent of the total output of magnesium was via sweat; the loss amounted to about 17 mg per day. Acclimatization did not occur, as it did in the cases of sodium and potassium. They concluded that under extreme conditions sweat could account for 25 per cent of the magnesium lost daily, and could be important when intakes are low.

By analogy with potassium, the other intracellular bulk cation, it is likely that there are mechanisms for maintenance of optimal cellular concentrations of magnesium at the cell membrane or within it. Maintenance of an optimal serum concentration is governed by intestinal absorption and renal excretion. Therefore, there are probably two homeostatic mechanisms for magnesium, one for the cell and one for the whole body.

There is indirect evidence that the adrenal cortex may partly govern magnesium homeostasis, as it does homeostasis of other major cationic electrolytes.

In hyperactivity magnesium levels may be low and in adrenal insufficiency high [7], mirroring potassium and contrary to sodium. Administration of aldosterone produces excretion of magnesium. The other endocrine organ affecting magnesium is the parathyroid, parathormone either acting directly on magnesium or through its action on calcium [7]. It is difficult to believe that this hormone governs cellular homeostasis, although it may govern bone stores.

4. *Balance of magnesium in man*

Most studies on magnesium balances in man have shown that an intake of 6 mg per kg body weight (420 mg for a 70 kg man) is necessary to achieve equilibrium and that intakes of less than this amount result in negative balances [4, 5]. JONES, *et al.* [19], however, found that less than 3 mg per kg caused negative balances and 3.6–4.2 mg per kg were adequate for positive balances during a week's time. To conclude that a negative balance of any major essential element exists throughout life is unrealistic and inconsistent with health and survival. For example, a small daily loss

of 10 mg of magnesium would deplete the total body content of 20 g in 5.5 yr; contrariwise, a positive balance of the same amount would double it. Therefore, we must assume that the long-term balance of intake and output of magnesium is equal, as it must be for sodium and potassium, and, during most of life, for calcium. Only during growth is the balance positive and during degeneration, negative. If 420 mg [4], or 350 mg [5] or 250 mg per day [19] are required for equilibrium, it is probable from our analyses that not many American adults ingest that much in their diets. MANOLO *et al.* [37] have shown that dietary magnesium and total calories are directly related according to regression analysis, and have suggested the formula Mg mEq $= -0.0117 + (0.0099 \times \text{calories})$. The slope of their regression line, however, would change in relation to the proportion of calories from fat and sugar in the diet, which contain virtually no magnesium. Most of their diets of 2000 cal or less contained less than 240 mg of magnesium, a level which they consider as marginal for equilibrium [19]. SHILS [38] on the other hand, put two men on a diet containing 8 mg magnesium per day for 117 and 266 days. Plasma and erythrocyte magnesium levels fell considerably and stabilized. Symptoms eventually occurred in both cases, relieved by 240 mg of magnesium intravenously. Although deficiency was produced, it was slow in appearing even under extreme conditions. JONES *et al.* [19] found that obese persons remained in balance on 1.8 mg per kg per day for short periods, whereas persons of normal weight required 3 mg per kg. The total amounts ingested, however, were similar. These findings are explicable by the relative aversion of magnesium to be stored in fat, and cast some doubt on the use of requirements for adults based on body weight.

The reason for the discrepancies between metabolic and balance studies may lie in methodology. The Titan yellow and similar colorimetric methods, so widely used, are influenced by the presence of calcium, aluminium, zinc, copper and tin, the brand of Titan yellow or thiazole dye, the type of magnesium for standards, and the concentration of sodium chloride [5, 39]. These variables were not fully appreciated until 1950 or later. It is possible that methodological errors of 10–20 per cent can explain the many negative balances listed by SEELIG [4] and HATHAWAY [5], almost all of which were reported prior to 1951. Modern analytical methods may resolve this discrepancy.

Balance studies appear to assume that a relatively fixed proportion of dietary magnesium is excreted in the stool, another proportion is excreted in the urine, and the amount absorbed by the intestine depends upon local factors, such as calcium, protein and vitamin D [4]. Such a system would depend upon chance mixtures of substances for homeostasis. Because the obligatory renal excretion of magnesium apparently is less than 1 mEq per day [28], in healthy people any magnesium in the urine in excess of 12 mg per day represents excesses not required by the body. When magnesium homeostasis is understood, the mechanism will probably be as simple as are homeostasis of sodium and potassium.

From this viewpoint, we can construct a tentative average balance for magnesium, based upon our data for intake and values from the literature for relative outputs in stool, urine and sweat.*

*By emission spectroscopy, balance studies were made on two subjects for 345 days and one for 35 days. Mean values (and ranges) per day (mg) were: Food, 290 (180–360); feces 210 (170–280); urine, 90 (72–98) total excreta, 300 (260–350); balance −10. In view of the many possible errors and compounding of errors in multiple analyses, we do not consider this balance negative. According to these data, 31 per cent of the magnesium ingested was excreted in the urine.

	Intake, mg		Output, mg
Food	210 (150–450)	Urine	80
Water	10 (0–50)	Stool	125
		Sweat	15 (0–55)
	220 (150–500)		220

The amount excreted in hair is negligible, about 7.5 μg daily.

This balance was based on 2 liters of water consumed in tea, coffee and other prepared drinks, at a hardness of 90 ppm containing 5 mg magnesium. As much as 120 mg would be supplied by the hardest municipal water in the United States [17], but it would not be very potable.

According to these concepts, the requirements for dietary magnesium by the healthy human adult are equal to obligatory losses by urine and sweat, just as are the requirements for sodium, potassium and calcium. The requirements for the growing child are larger on a weight basis; for every kg of muscle produced, about 200 mg of magnesium must be provided, and for every kg increase of body weight, about 300 mg in excess of obligatory losses. As infants have retained magnesium on intakes of 3.5 mg per kg per day [5] it is doubtful that ordinary infant diets are deficient. Similarly, it is doubtful that adult diets are deficient, unless subjects are suffering from one of several severe diseases.

5. *Magnesium deficiency in man*

WACKER and PARISI [7] have contributed an excellent review of disturbances of magnesium metabolism. Elevated serum levels are accompanied by depression of the sensorium, and are found in uremia. Hypomagnesemia occurs in a number of conditions, resulting in neuromuscular excitability, tetany and convulsions. Evidence that cellular stores of magnesium are seriously depleted in extracellular depletion is largely lacking. Serum contains about 1.2 mEq of ionic magnesium per l., and extracellular fluid probably has the same concentration. In 20 l. there would be 288 mg, about 1.4 per cent of the total body content. Red blood cells contain about 150 mg and protein in serum 19 mg, all of it bound. Loss of 200 mg of extracellular magnesium without replacement from readily mobilizible stores would result in low serum levels and might be accompanied by hypomagnesemic tetany. As there are 11 g of magnesium in bone alone, extracellular fluid magnesium represents only 2.6 per cent of this one store. It is possible, therefore, that hypomagnesemia with symptoms may occur with little or no depletion of soft tissue magnesium. The fact that replacement of the extracellular deficit by parenteral injection will restore serum levels and relieve symptoms suggests that this may be true. It is apparent that severe depletion of cellular magnesium could result in cessation of oxidative phosphorylation.

Hypomagnesemia due to poor absorption occurs in intestinal malabsorption from many disorders of the small bowel. Steatorrhea can remove magnesium, as can excessive diarrhea, continuous gastric suction, malnutrition and other gastrointestinal diseases.

Excess urinary excretion can also cause extracellular magnesium deficiency in severe renal diseases, a finding reminiscent of "salt-losing nephritis". The chronically diseased kidney has the capacity "to maintain a normal absolute rate of magnesium

excretion per nephron until far advanced disease supervenes", according to STEELE *et al.* [40], after which time hypermagnesemia results. The converse experiment, the ability of the diseased kidney to conserve magnesium, has not been ascertained, but it probably deteriorates along with other tubular mechanisms. Excessive use of diuretics can also lead to depletion. Alcoholism is frequently associated with low serum magnesium levels, partly because of low intakes in terms of caloric requirements, partly because of excess urinary excretion by some unknown mechanism [41].

Cellular magnesium depletion probably occurs in disorders involving loss of cellular potassium. Hyponatremia, acidosis and dehydration interfere with the integrity of the cellular membrane, resulting in losses of intracellular cations and penetration of the cell by extracellular ones. Shock, diabetic acidosis, overhydration or sodium depletion and other changes in electrolytes may be associated with true magnesium deficiency, but these disorders are not long lasting.

Certain endocrine diseases are associated with hypomagnesemia and may be accompanied by loss of magnesium stores. Hyperparathyroidism is accompanied by bodily losses of magnesium (from bone?); retention occurs when the disorder is corrected. Hyperfunction of the adrenal cortex is often associated with magnesium loss. In hyperthyroidism, low plasma and high urinary levels with normal exchangeable magnesium have been found; in hypothyroidism exhangeable magnesium was low [42].

Convulsive disorders of newborn infants can result from magnesium deficiency from unknown cause and prevented by magnesium supplements [43]. It is not known whether or not the diets of the mothers were deficient prior to delivery.

6. *Is magnesium the water factor?*

Some variable in, or associated with, potable municipal water and linked to hardness of water is inversely related to regional and municipal death rates from cardiovascular disease in the United States [44, 45], England and Wales [46, 47] and Sweden [48]. First discovered in 1960 [44], it was based on the findings of KOBAYASHI in Japan that cerebral hemorrhage was associated with an elevated sulfate: bicarbonate ratio of river water according to region [49]. Japanese river waters are soft and therefore low in magnesium and calcium [50]; hardness of water is not concerned with this relationship in that country [45]. In British and Swedish waters, cardiovascular death rates were inversely related to concentrations of calcium and hardness but not of magnesium [46]; in the United States, magnesium was a factor in the association [45].

Based on this finding, NEAL and NEAL [25] gave cholesterol-fed rabbits hard and soft waters and waters with and without magnesium. The hard and magnesium containing waters protected them partly or completely against aortic atherosclerosis. Furthermore, CRAWFORD and CRAWFORD [51] found less magnesium in the coronary arteries of persons dying of atherosclerotic heart disease in soft than in hard water areas.

If there is a single factor in water affecting cardiovascular death rates favorably, epidemiological evidence suggests that it is not magnesium. The increment added by water to the total dietary intake is small [26, 45], except in very hard water areas, and could scarcely make the difference between marginal and adequate intakes of magnesium in view of this discussion. Experimental magnesium deficiency produces aortic and renal calcification [4], but the evidence that it produces typical athero-

sclerosis is lacking. Calcification is merely the end stage of atheroma formation. Whatever the water factor is, it must act in otherwise healthy people in whom atherosclerosis slowly develops; magnesium deficiency is as inconsistent with good health as is potassium, calcium, or sodium deficiency.

7. *Magnesium and chronic diseases*

Granted that dietary magnesium deficiency does not occur in reasonably healthy persons, are there any major chronic diseases of man of unknown cause which might result from an imbalance of magnesium, calcium and other elements? If there are, they are likely to be concerned with calcification or decalcification. Experimentally, magnesium, calcium, phosphate and fluoride have been considered to show interrelations; magnesium deficiency has resulted in metastatic calcification which was prevented by fluoride [27].

Concentration gradients across the cellular membrane of extracellular calcium and intracellular magnesium are as basic to cellular function as are those of sodium and potassium [52]; ratios of monovalent and divalent cations are related linearly. Whereas these gradients may be altered in disease, they are interdependent, and a change in one is accompanied by a change in the others. Therefore, it is doubtful that cellular disorders are the result of magnesium deficiency or excess alone.

We are aware of no good relationship of any major chronic disease of unknown etiology being associated with any of the disorders accompanied by hypomagnesemia. If magnesium imbalance occurs in a disorder, it is more likely to be secondary to the disorder, and not causal, just as other electrolyte imbalances are secondary.

Therefore, clinically magnesium should not be considered as an entity separate from other electrolytes, but should be included whenever electrolyte disturbances are evaluated.

Conditions which involve alterations in extracellular electrolytes, and which lead to depletion of cellular potassium, should always be examined for concomitant changes in magnesium concentrations in erythrocytes, serum and urine.

SUMMARY AND CONCLUSIONS

Analyses of tissues from 399 foreign and United States subjects were made by emission spectroscopy for magnesium. Many common foods and a few waters were analyzed by atomic absorption spectrophotometry.

Although some tissues of infants and young children had slightly higher concentrations of magnesium than did those of adults, mean concentrations from the second to the ninth decades of life were remarkably constant in muscle, pancreas, spleen, liver, kidney, heart, lung and brain. Magnesium and calcium accumulated progressively in human aortas with age, and presumably atherosclerotic disease. No essential differences were found in concentrations of magnesium in American and foreign tissues.

Levels of magnesium in tissues were unremarkable in subjects dying of uremia or chronic alcoholism. When tissues were low or high in magnesium, no correlation with any specific disease was found.

Concentrations of magnesium in 29 tissues were frequently related to those of manganese and phosphorus, rank correlations being positive, less frequently to

copper, zinc and iron, and infrequently to chromium, calcium, cobalt and potassium. They were sometimes related to barium and aluminum.

Bone contained 55 per cent and muscle 26.5 per cent of the total body burden of magnesium, occurring at the highest concentrations. Major organs, except lung, had moderate concentrations; the lowest were found in fat, blood, skin, omentum and adrenal.

Foods containing the largest amounts of magnesium on a weight basis were nuts, cereals and whole grains, sea foods, meats and legumes. Intermediate levels occurred in green vegetables, dairy products and fruits. Fats and refined sugars were almost devoid of magnesium. Refining of foods by fractionation resulted in large losses of magnesium, especially in sugars and grains. Boiling of vegetables caused major losses into water. Animal feeds contained 5–14 times as much magnesium as most human foods.

Diets of 110–650 mg magnesium per day could be formulated from these data without deviating from individual habit patterns in this country. Two institutional and hospital diets contained 200–220 mg magnesium, which for a 70 kg individual would supply about 3 mg per kg body weight; this value has been said to produce negative balances. It is unlikely that negative balances occur for long periods of time.

Hypomagnesemia is associated with neuromuscular excitability and hypermagnesemia with depression of the sensorium. It is probable that severe depletion of intracellular magnesium is inconsistent with life, and that moderate depletion causes severe depression of cellular function involving phosphate energy exchanges. As one of the two major intracellular cations, magnesium is necessary for normal cellular function. Disturbances of magnesium metabolism should probably be considered with disturbances of other major electrolytes.

There is no evidence of widespread dietary magnesium deficiency, except in states of malabsorption and excessive excretion due to other conditions. Magnesium intake appears to be adequate, probably because of efficient renal homeostatic mechanisms. The daily dietary requirement of magnesium for adult man in probably the obligatory urinary excretion plus losses in sweat and the amount unabsorbed in feces. There is no good evidence that marginal dietary intakes contribute to any chronic disease of unknown etiology.

REFERENCES

1. Mason, B.: *Principles of Geochemistry*. 2nd Ed. Wiley, New York, 1952.
2. McCann, F. V. and Wira, C. R.: Transmembrane ionic gradients in lepidoptera as related to cardiac electrical activity. *Comp. Biochem. Physiol.* 22, 611, 1967.
3. Kruse, H. D., Orent, E. R. and McCollum, E. V.: Studies on magnesium deficiency in animals—I. Symptomatology resulting from magnesium deprivation. *J. Biol. Chem.* 96, 519, 1932.
4. Seelig, M. S.: The requirement of magnesium by the normal adult. *Am. J. Clin. Nutr.* 14, 342, 1964.
5. Hathaway, M. L.: Homes Economics Research Report No. 19, Agricultural Research Service, *Magnesium in Human Nutrition*. U.S. Dept. of Agriculture, U.S. Government Printing Office, Washington, 1962.
6. Wacker, W. E. C. and Vallee, B. L.: Magnesium in *Mineral Metabolism*, Vol. 2A (Comar, C. L. and Bronner, F, Eds.). Academic, New York, 1964.
7. Wacker, W. E. C. and Parisi, A. F.: Magnesium metabolism. *New Eng. J. Med.* 278, 658, 1968.
8. Barker, E. S.: Physiologic and clinical aspects of magnesium metabolism. *J. chron. Dis.* 12, 278, 1960.

9. TIPTON, I. H., COOK, M. J., STEINER, R. L., BOYE, C. A., PERRY, H. M., JR. and SCHROEDER H. A.: Trace elements in human tissue—I. Methods. *Health Physics* 9, 89, 1963.
10. TIPTON, I. H. and COOK, M. J.: Trace elements in human tissue—II. Adult subjects from the United States. *Health Physics* 9, 103, 1963.
11. TIPTON, I. H., SCHROEDER, H. A., PERRY, H. M., JR. and COOK, M. J.: Trace elements in human tissue—III. Subjects from Africa, the Near and Far East and Europe. *Health Physics* 11, 403, 1965.
12. SCHROEDER, H. A., NASON, A. P., TIPTON, I. H. and BALASSA, J. J.: Essential trace metals in man: Zinc. Relation to environmental cadmium. *J. chron. Dis.* 20, 179, 1967.
13. FISHER, G. M., MATA, E. I. and LLAURADO, J. G.: Regional differences in magnesium, calcium and zinc composition of arterial wall in normal and hypertensive dogs. *Am. Heart J.* 75, 784, 1968.
14. Agricultural Research Service, *Composition of Foods*. Agricultural Handbook No. 8, U.S. Department of Agriculture, U.S. Government Printing Office, Washington, 1963.
15. SCHROEDER, H. A.: The role of chromium in mammalian nutrition. *Am. J. Clin. Nutr.* 21, 230, 1968.
16. CZERNIEJEWSKI, C. P., SHANK, C. W., BECHTEL, W. G. and BRADLEY, W. B.: The minerals of wheat, flour and bread. *Cereal Chem.* 41, 65, 1964.
17. DURTOR, C. N. and BECKER, E.: *Public water supplies of the 100 largest cities in the United States*, 1962, U.S. Geol. Surv. Water-Supply Paper No. 1812, U.S. Government Printing Office, Washington, 1964.
18. MARHER, J. R.: Some aspects of magnesium metabolism with particular reference to humans. *Int. J. Vitalstoff. Zivilisationskrankheiten* (In press).
19. JONES, J. E., MANALO, R. and FLINK, E. B.: Magnesium requirements in adults. *Am. J. clin. Invest.* 20, 632, 1967.
20. DIXON, M. and WEBB, E. C.: *Enzymes*. 2nd Edn. Academic, New York, 1964.
21. SCHROEDER, H. A., NASON, A. P., TIPTON, I. H. and BALASSA, J. J.: Essential trace metals in man: Copper. *J. chron. Dis.* 19, 1007, 1966.
22. SCHROEDER, H. A., BALASSA, J. J. and TIPTON, I. H.: Essential trace metals in man: Manganese: A study in homeostasis. *J. chron. Dis.* 19, 545, 1966.
23. SCHROEDER, H. A., BALASSA, J. J. and TIPTON, I. H.: Abnormal trace metals in man. Chromium *J. chron. Dis.* 15, 941, 1962.
24. BORST, J. G. G.: Protein katabolism in uremia; effects of protein-free diet; infections and blood transfusion. *Lancet* i, 824, 1948.
25. NEAL, J. B. and NEAL, M.: Effect of hard water and MgSO on rabbit atherosclerosis. *Archs Path.* 73, 58, 1962.
26. GOLDSMITH, N. F. and GOLDSMITH, J. R.: Epidemiological aspects of magnesium and calcium metabolism. *Archs Environ. Hlth* 12, 607, 1966.
27. HEGSTED, D. M.: Mineral intake and bone loss. *Fed. Proc.* 26, 1747, 1967.
28. BARNES, B. A., COPE, O. and HARRISON, T.: Magnesium conservation in human being on low magnesium diet. *J. clin. Invest.* 37, 430, 1958.
29. *Stability Constants of Metal-ion Complexes*, The Chemical Society, Burlington House, London 1964.
30. GRAHAM, L. A., CAESAR, J. J. and BURGER, A. S. V.: Gastrointestinal absorption and excretion of ^{28}Mg in man. *Metabolism* 9, 646, 1960.
31. SILVER, L., ROBERTSON, J. S. and DAHL, L. K.: Magnesium turnover in the human studied with ^{28}Mg. *J. clin. Invest.* 39, 420, 1960.
32. *Documenta Geigy*. Scientific Tables, Diem, K. ed. 6th Ed. Geigy Pharmaceuticals, Ardsley, N.Y. 1962.
33. ALCOCK, N. W., MACINTYRE, I. and RADDE, I.: Concentration of magnesium in human plasma or serum. *Nature, Lond.* 206, 89, 1965.
34. FARNSWORTH, E. B. and KRAKUSIN, J. S.: Electrolyte partition in patients with edema of various origins. *J. Lab. Clin. Med.* 33, 1534, 1948.
35. FUTCHER, P. H. and SCHROEDER, H. A.: Studies on congestive heart failure—II. Impaired renal excretion of sodium chloride. *Am. J. med. Soc.* 204, 52, 1942.
36. CONSOLAZIO, C. F., MATOUSH, L. O., NELSON, R. A., HARDING, R. S. and CANHAM, J. E.: Excretion of sodium potassium, magnesium and iron in human sweat and the relation of each to balance and requirements. *J. Nutr.* 79, 407, 1963.
37. MANOLO, R., FLORA, R. E. and JONES, J. E.: A simple method for estimating dietary magnesium. *Am. J. Clin. Nutr.* 20, 627, 1967.
38. SHILS, M. E.: Experimental human magnesium depletion. *Am. J. clin. Nutr.* 15, 133, 1964.

39. SANDELL, E. B.: *Colorimetric determination of traces of metals* 3rd Ed. Interscience, New York, 1959.
40. SELLE, T. H., WENS, S., EVENSON, M. A. and RIESELBACH, R. E.: The contribution of the chronically diseased kidney to magnesium homeostasis in man. *J. Lab. clin. Med.* 71, 455, 1968.
41. KALBFLEISCH, J. M., LINDEMAN, R. D., GINN, H. E. and SMITH, W. O.: Effects of ethanol administration on urinary excretion of magnesium and other electrolytes in alcoholic and normal subjects. *J. clin. Invest.* 42, 1471, 1963.
42. JONES, J. E., DESPER, P. C., SHANE, S. R. and FLINK, E.B..: Magnesium metabolism in hyperthyroidism and hypothyroidism. *J. clin. Invest.* 45, 891, 1966.
43. WONG, H. B. and TEH, Y. F.: An association between serum-magnesium and tremor and convulsions in infants and children. *Lancet* ii, 18, 1968.
44. SCHROEDER, H. A.: Relations between hardness of water and death rates from certain chronic and degenerative diseases in the U.S. *J. chron. Dis.* 12, 586, 1960.
45. SCHROEDER, H. A.: Municipal drinking water and cardiovascular death rates. *J. Am. med. Soc.* 195, 81, 1966.
46. MORRIS, J., CRAWFORD, M. D. and HEADY, J. A.: Hardness of local water supplies and mortality from cardiovascular disease. *Lancet* i, 860, 1961.
47. CRAWFORD, M. D., GARDNER, M. J. and MORRIS, J. N.: Mortality and hardness of local water-supplies. *Lancet* i, 827, 1968.
48. BIORCK, G., BOSTROM, H. and WIDSTROM, A.: On the relationship between water hardness and death rates in cardiovascular diseases. *Acta med. scand.* 178, 239, 1965.
49. KOBAYASHI, J.: Geographical relationship between chemical nature of river water and death-rate from apoplexy. *Berichte d Ohara Inst. landwirtsch. Biologie* 11, 12, 1957.
50. KOBAYASHI, J.: Chemical study of the average quality and characteristics of river waters of Japan. *Berichte d Ohara Inst. landwirtsch. Biologie* 11, 313, 1960.
51. CRAWFORD, T. and CRAWFORD, M. D.: Prevalence and pathological changes of ischaemic heart-disease in a hard-water and in a soft-water area. *Lancet* i, 229, 1967.
52. WILLIAMS, R. J. P. and WACKER, W. E. C.: Cation balance in biological systems. *J. Am. med. Soc.* 201, 18, 1967.
53. LOWE, K. G.: Metabolic studies with protein-free electrolyte-free diet in man. *Clin. Science* 12, 57, 1953.
54. SHILS, M. E.: Experimental production of magnesium deficiency in man. *Ann. N. Y. Acad. Sci* (in press).
55. CHESLEY, L. C. and TEPPER, I.: Some effects of magnesium loading upon renal excretion of magnesium and certain other electrolytes. *J. clin. Inves.* 37, 1362, 1958.

Naturwissenschaften,
46:385-6, 1959.

Magnesium Tetany and Toxicity of Various Magnesium Salt Solutions upon Intraperitoneal Injection

by

O. Selisko and H. Ackermann

According to McCollum et al. (1,2) and Syllm-Rapoport and Strassburger (3), genuine tetany can be elicited experimentally in rats and dogs with a diet defficient in magnesium. Our experiments show that the intraperitoneal application of high doses of magnesium to albino rats also elicits symptoms of tetany*.

The calcium level remains practically normal and it may thus be assumed that the convulsions observed were due merely to the sudden shift of the calcium:magnesium quotient. There is also the possibility of a relative hypocalcemia caused by the administration of high doses of magnesium.

The toxicity of aqueous solutions of magnesium salts upon intraperitoneal administration largely depends upon the anion

---

* After termination of our experiments we learned of the report of Hartmann, Wiederholt and Schreier (Naturwissen. 46:112, 1959) in which the authors described seizures and death after the i.p. administration of 0.5 mg magnesium/g body weight.

and the concentration. We have tested ascorbate, chloride, acetate, thiosulfate, sulfate and adipinate in 3 concentrations (in the case of ascorbate only in 2 concentrations for reasons of solubility). In every case the same quantity of magnesium was applied, namely 9.75 mg. All the animals (inbred albino rats) weighed 95±5 g. The injections were administered intraperitoneally within 2-3 sec.

The intensity of the convulsions was far greater with salts of monobasic acids than those of dibasic acids. The number of deaths clearly underscored this observation. It also showed that with the three first preparations the toxicity increases with the rise in concentration, while with the other two the medium concentration was the most toxic (no longer detectable in the case of adipinate).

The animals that survived the convulsions, when these were at an end, fell into the well-known magnesium narcosis, from which they recovered relatively rapidly and completely. This narcosis developed in all cases, including those in which there had been no prior signs of convulsions. Deep narcosis can be produced without a prior state of excitation even with small doses. We therefore believe that the convulsions should be regarded not as a stage of excitation, but as a symptom of tetany. A chronic experiment would be of interest, but fails owing to the rapid excretion of magnesium.

Orientating experiments have shown that for instance

acetate is more rapidly absorbed than adipinate. This fact would appear to be primarily responsible for the variable effect of magnesium salts.

A more detailed report will follow on these and other studies on the effect of magnesium. We thank the Scharffenberg Company, Dresden, E. Merck, Darmstadt and Nordmarkwerke, Hamburg, for their kind gift of magnesium preparations.

Table

Ergebnisse Results

1 Salz	2 Konzentration	3 Tierzahl	4 Krämpfe bei	5 Exitus
Ascorbinat	a	100	74 = 74%	27 = 27%
Ascorbinat	b	45	44 = 98%	31 = 69%
Chlorid	a	30	11 = 37%	1 = 3%
Chlorid	b	30	30 = 100%	25 = 83%
Chlorid	c	30	30 = 100%	30 = 100%
Acetat	a	30	14 = 47%	1 = 3%
Acetat	b	40	[illegible]	25 = 62%
Acetat	c	30	30 = 100%	30 = 100%
Thiosulfat	a	70	8 = 11%	2 = 3%
Thiosulfat	b	85	67 = 79%	26 = 31%
Thiosulfat	c	60	22 = 37%	6 = 10%
Sulfat	a	40	3 = 7%	0 = 0%
Sulfat	b	30	22 = 73%	3 = 10%
Sulfat	c	30	18 = 60%	2 = 7%
Adipinat	a	20	0 = 0%	0 = 0%
Adipinat	b	20	0 = 0%	0 = 0%
Adipinat	c	20	0 = 0%	0 = 0%

6 Mg-Dosis je 9,75 mg: a) 1,5 ml mit 6,5 mg Mg/ml. — b) 0,75 ml mit 13,0 mg Mg/ml. — c) 0,55 ml mit 17,8 mg Mg/ml.

Key: 1. Salt. 2. Concentration. 3. Number of animals. 4. Convulsions in. 5. Death. 6. Mg dose 9.75 mg each:a) 1.5 ml with 6.5 mg Mg/ml. b) 0.75 ml with 13.0 mg Mg/ml. c) 0.55 ml with 17.8 mg Mg/ml.

Bibliography.

See p. 386

Naturwissenschaften (1950) 38: 385-386

Über die „Magnesiumtetanie" und die Toxizität verschiedener Magnesiumsalzlösungen bei intraperitonealer Injektion

Nach McCollum et al.[1],[2]) sowie Sylim-Rapoport und Strassburger[3]) kann an Ratten und Hunden durch Magnesiummangeldiät experimentell eine echte Tetanie erzeugt werden. Unsere Versuche zeigten, daß die intraperitoneale Applikation hoher Magnesiumdosen bei Albinoratten ebenfalls die Symptome einer Tetanie auslöst*). Der Calciumspiegel bleibt dabei praktisch normal, und es ist daher anzunehmen, daß die beobachteten Krampfanfälle lediglich auf die plötzliche Verschiebung des Quotienten Calcium:Magnesium zurückzuführen sind. Es könnte dabei also an eine durch die Zufuhr hoher Magnesiumgaben hervorgerufene relative Hypocalcämie gedacht werden.

Die Toxizität wäßriger Magnesiumsalzlösungen ist bei i.p.-Gabe weitgehend vom Anion und von der Konzentration abhängig. Wir prüften Ascorbinat, Chlorid, Acetat, Thiosulfat, Sulfat und Adipinat in je 3 (bei Ascorbinat aus Löslichkeitsgründen nur 2) Konzentrationen. Appliziert wurde in jedem Falle die gleiche *Magnesium*-Menge, nämlich 9,75 mg. Alle Tiere (Inzucht-Albinoratten) wogen je 95 ± 5 g. Die Injektionen erfolgten in 2 bis 3 sec intraperitoneal.

Ergebnisse

Salz	Konzentration	Tierzahl	Krämpfe bei	Exitus
Ascorbinat . . .	a	100	74 = 74 %	27 = 27 %
Ascorbinat . . .	b	45	44 = 98 %	31 = 69 %
Chlorid	a	30	11 = 37 %	1 = 3 %
Chlorid	b	30	30 = 100 %	25 = 83 %
Chlorid	c	30	30 = 100 %	30 = 100 %
Acetat	a	30	14 = 47 %	1 = 3 %
Acetat	b	40	40 = 100 %	25 = 62 %
Acetat	c	30	30 = 100 %	30 = 100 %
Thiosulfat	a	70	8 = 11 %	2 = 3 %
Thiosulfat	b	85	67 = 79 %	26 = 31 %
Thiosulfat	c	60	22 = 37 %	6 = 10 %
Sulfat	a	40	3 = 7 %	0 = 0 %
Sulfat	b	30	22 = 73 %	3 = 10 %
Sulfat	c	30	18 = 60 %	2 = 7 %
Adipinat	a	20	0 = 0 %	0 = 0 %
Adipinat	b	20	0 = 0 %	0 = 0 %
Adipinat	c	20	0 = 0 %	0 = 0 %

Mg-Dosis je 9,75 mg: a) 1,5 ml mit 6,5 mg Mg/ml. — b) 0,75 ml mit 13,0 mg Mg/ml. — c) 0,55 ml mit 17,8 mg Mg/ml.

Die Intensität der Krampfanfälle war bei Anwendung der Salze einbasischer Säuren weit größer als bei denen der zweibasischen. Die Anzahl der Exitus unterstreicht diese Beobachtung eindeutig. Sie zeigt ferner, daß bei den drei erstgenannten

Präparaten die Toxizität mit der Konzentration steigt, während bei den drei anderen die mittlere Konzentration am meisten toxisch wirkt (bei Adipinat nicht mehr nachweisbar).

Die Tiere, welche die Krämpfe überlebten, fielen nach deren Abklingen in die bekannte Magnesiumnarkose, aus der sie sich relativ rasch und vollständig erholten. Diese Narkose trat in allen Fällen auf, also auch dann, wenn keine Krampferscheinungen vorausgingen. Auch mit geringeren Dosen läßt sich tiefe Narkose ohne vorherigen Erregungszustand erzielen. Daher glauben wir, daß die Krämpfe nicht als Exzitationsstadium, sondern als Symptom einer Tetanie zu deuten sind. Der chronische Versuch wäre von Interesse, er scheitert jedoch an der raschen Ausscheidung des Magnesiums.

Durch orientierende Versuche konnten wir nachweisen, daß z.B. das Acetat viel schneller resorbiert wird als das Adipinat. Diese Tatsache scheint also vorwiegend für die unterschiedliche Wirkung der Magnesiumsalze verantwortlich zu sein.

Wir werden über diese und weitere Untersuchungen auf dem Gebiet der Magnesiumwirkung noch ausführlicher berichten. Wir danken den Firmen Scharffenberg, Dresden, E. Merck, Darmstadt, und Nordmarkwerke, Hamburg, für die freundliche Überlassung von Magnesiumpräparaten.

Deutsche Akademie der Wissenschaften zu Berlin, Institut für Ernährung, Potsdam-Rehbrücke

O. Selisko und H. Ackermann

Naturwissenschaften,
50:13, 479-80, 1963.

Mean Lethal Dose (LD_{50}) of Some Magnesium Salts in Aqueous Solutions

by

O. Selisko and H. Ackermann

A report was published earlier (1) on the influence of the concentration of aqueous magnesium salt solutions on their acute toxicity, but no numerical data were given for the LD_{50} of these preparations. There was reason for this, for in the mean time in a large-scale experiment reported elsewhere (2), we have studied the extent to which the LD_{50} is shifted and the exogenous factors that cause the shift when the experimental conditions that are under the influence of the experimentator are kept as constant as possible. It was found that changes in relative humidity of the air in the room could be regarded as the direct cause of enormous variations in the LD_{50}. We were unaware of this when we reported the LD_{50} values of Mg salts cited below. Our reason for publishing them nevertheless is that subsequent information received from the Main Office of Climatology (Potsdam) revealed that at the time of these determinations there had been no appreciable changes in climatic conditions. At the same time the values were in good accord with those found earlier (1) for the variable effect of the

same magnesium salts in experimental tetany. As is known, the therapeutic - and thus also the toxic - effect of parenterally applied Mg preparations is due solely to the rise in the magnesium level in the blood and is thus caused solely by the cation. Thus if we wish to measure the LD_{50} of several Mg salts comparatively, it is best to adjust the solutions in such a way that the corresponding concentrations will have the same magnesium content. As before (1), we selected "Magnorbin" (E. Merck) and "Magnosulf" (Nordmark-Werke) commercial preparations, as well as the analytically pure salts Mg chloride, Mg acetate, Mg sulfate and Mg adipinate. 10% Magnorbin (Mg ascorbate) contains 6.5 mg magnesium and thus 20% Magnorbin contains 13.0 mg Mg/ml. Magnosulf (Mg thiosulfate) contains 17.8 mg Mg/ml. By dilution we obtained a concentration of 6.5 and 13.0 with this preparation as well. Three solutions were made with each of the other four Mg salts with analogous Mg values (6.5, 13.0, and 17.8 mg Mg/ml). The 17.8 concentration cannot be produced from Mg ascorbate for reasons of solubility.

The LD_{50} of the preparations was determined on Wistar albino rats from our own strain (3) according to the method of Kärber (4). The animals (male and female) weighed 100±5 g. Number of groups for each determination: 5. Number of animals per group: 8. Application was in the form of a single i.p. injection within 2-3 sec. Dosages ranged from 0.1 to 0.25 ml.

Duration of observation was 24 hours. Season: mid-May. The table shows the LD_{50} values (in mg Mg/kg body weight) for solutions of different Mg salts and different concentrations (in mg Mg/ml).

All the values given mean the LD_{50} in mg magnesium per kg body weight, in other words not salt quantities. As can be seen, the salts of monobasic acids are more toxic than those of dibasic acids. Furthermore the toxicity increases with rising concentration, the small deviations being negligible. All this shows that the speed of absorption alone may be regarded as the cause of the differences in the effect of the preparations. The degree of dissociation scarcely plays a role, since with the relatively weak aqueous solutions almost complete dissociation is assumed. Determinations of the activity coefficient did not give any significant indication of the differences in the LD_{50} values, which, as can be seen, are very considerable. It should be noted that the experiments with Mg adipinate had to be repeated several times, for this salt has such a low toxicity that it was impossible to administer lethal doses in a few applications unless disproportionately large volumes were administered. These values in the table (200, 180, 160) thus undoubtedly represent the lowest limits of these LD_{50}.

In accordance with our views on the desirable degree of accuracy of calculations of toxicological values we give them in rounded figures at two important points.

We thank Dr. G. Gebhardt, Freiberg i. Sa., for the determination of the activity coefficients. We also thank the Scharffenberg Company, Dresden, E. Merck, Darmstadt and Nordmark-Werke, Hamburg for their kind gift of preparations.

Table.

1 Konzentration	6,5	13,0	17,8	Konzentration	6,5	13,0	17,8
2 Mg-Ascorbinat.	100	88	—	Mg-Thiosulfat	120	100	110
3 Mg-Chlorid . .	100	90	66	Mg-Sulfat . .	150	130	140
4 Mg-Acetat . .	100	100	73	Mg-Adipinat	200	180	160

Key: 1. Concentration. 2. Mg ascorbate. 3. Mg chloride. 4. Mg acetate.

Bibliography

See p. 480.

From: German Academy of Sciences, Berlin, Institute of Nutrition, Potsdam-Rehbrücke.

Sent in: Feb. 18, 1963.

Naturwissenschaften
50: 12, 479-480 (1963)

Über die „mittlere tödliche Dosis" (LD_{50}) einiger Magnesiumsalze in wäßriger Lösung

Wir haben schon früher[1]) über den Einfluß der Konzentration wäßriger Magnesiumsalzlösungen auf ihre akute Toxizität berichtet, gaben jedoch noch keine zahlenmäßigen Werte für die LD_{50} dieser Präparate an. Das hatte seinen Grund. Denn wir studierten inzwischen in einem Großversuch, über den an anderer Stelle[2]) berichtet wird, in welchem Ausmaß und durch welche *exogenen* Faktoren die LD_{50} verschoben wird, wenn gleichwohl die vom Experimentator beeinflußbaren Versuchsbedingungen so konstant wie möglich gehalten werden. Dabei ergab sich, daß Veränderungen der relativen Luftfeuchtigkeit *im Versuchraum* als unmittelbare Ursache enormer Schwankungen der LD_{50} anzusehen sind. Das war uns, als wir die nachstehend mitgeteilten Werte der LD_{50} von Mg-Salzen ermittelten, noch nicht bekannt. Wenn wir sie dennoch publizieren, so deshalb, weil nachträgliche Informationen beim Hauptamt für Klimatologie (Potsdam) ergaben, daß zur Zeit jener Bestimmungen keine nennenswerten Veränderungen der klimatischen Gegebenheiten auftraten. Zum andern korrespondieren die Werte gut mit denen, die wir seinerzeit[1]) für die unterschiedliche Wirkungsintensität der gleichen Mg-Salze bei experimentell erzeugter Tetanie gefunden haben. Bekanntlich beruht der therapeutische — und damit auch der toxische — Effekt parenteral applizierter Mg-Präparate allein auf dem Anstieg des Magnesiumspiegels im Blut, ist mithin ausschließlich durch das Kation bedingt. Will man also die LD_{50} mehrerer Mg-Salze vergleichend

messen, dann ist es zweckmäßig, ihre Lösungen so einzustellen, daß die korrespondierenden Konzentrationen den gleichen *Magnesium*-Gehalt aufweisen. Wie früher[1]) wählten wir die Handelspräparate „Magnorbin" (E. Merck) und „Magnosulf" (Nordmark-Werke), ferner die analysenreinen Salze Mg-Chlorid, Mg-Acetat, Mg-Sulfat und Mg-Adipinat. Magnorbin (Mg-Ascorbinat) 10% enthält je ml 6,5 mg Magnesium, Magnorbin 20%, daher 13,0 mg Mg/ml. Magnosulf (Mg-Thiosulfat) enthält 17,8 mg Mg/ml. Durch Verdünnen erhielten wir auch davon die Konzentrationen 6,5 und 13,0. Von den anderen vier Mg-Salzen wurden je drei Lösungen mit den analogen Mg-Gehaltwerten (6,5, 13,0 und 17,8 mg Mg/ml) hergestellt. Die Konzentration 17,8 ist von Mg-Ascorbinat aus Löslichkeitsgründen nicht herstellbar.

Die LD_{50} der Präparate wurde an Albinoratten (Wistar) eigener Zucht[3]) nach der Methode von KÄRBER[4]) bestimmt. Die Tiere (männlich und weiblich) wogen je 100 ± 5 g. Anzahl der Gruppen je Bestimmung: 5. Anzahl der Tiere je Gruppe: 8. Applikation i.p. in 2 bis 3 sec, einmalige Gabe. Dosisintervalle 0,1 bis 0,25 ml. Beobachtungsdauer 24 Std. Jahreszeit: Mitte Mai. Die Tabelle gibt die Werte LD_{50} (in mg Mg/kg Gew.) für Lösungen verschiedener Mg-Salze und verschiedene Konzentrationen (in mg Mg/ml) an.

Konzentration	6,5	13,0	17,8	Konzentration	6,5	13,0	17,8
Mg-Ascorbinat .	100	88	—	Mg-Thiosulfat	120	100	110
Mg-Chlorid . .	100	90	66	Mg-Sulfat . .	150	130	140
Mg-Acetat . .	100	100	73	Mg-Adipinat	200	180	160

Alle angegebenen Zahlen bedeuten die jeweilige LD_{50} in *Milligramm Magnesium* je Kilogramm Körpergewicht, also nicht Salzmengen. Wie ersichtlich, sind die Salze der einbasischen Säuren toxischer als die der zweibasischen. Außerdem steigt die Toxizität mit der Konzentration, wobei kleine Abweichungen ohne Belang sind. Das alles zeigt, daß die Resorptionsgeschwindigkeit allein als Ursache für die unterschiedliche Wirksamkeit der Präparate angesehen werden darf. Der Dissoziationsgrad spielt dafür kaum eine Rolle, denn man kann bei diesen relativ schwachen, wäßrigen Lösungen praktisch vollständige Dissoziation annehmen. Auch Bestimmungen des Aktivitätskoeffizienten gaben keinen signifikanten Anhaltspunkt für die Verschiedenheiten der LD_{50}-Werte, die, wie man sieht, sehr beträchtlich sind. Es sei bemerkt, daß wir die Versuche mit Mg-Adipinat mehrfach wiederholen mußten. Denn dieses Salz ist so wenig toxisch, daß es einige Male nicht möglich war, letale Dosen zu applizieren, es sei denn, man hätte unverhältnismäßig hohe Volumina injiziert. Die betreffenden Tabellenwerte (200, 180 und 160) stellen somit sicherlich die untersten Grenzen dieser LD_{50} dar.

Es entspricht unserer Ansicht über die noch sinnvolle Berechnungsschärfe toxikologischer Meßwerte, daß wir sie abgerundet auf zwei werthabende Stellen angeben.

Wir danken Herrn Dr. G. GEBHARDT, Freiberg i. Sa., für die Bestimmung der Aktivitätskoeffizienten. Wir danken ferner den Firmen Scharffenberg, Dresden, E. Merck, Darmstadt, und Nordmark-Werke, Hamburg, für die freundliche Überlassung von Präparaten.

Deutsche Akademie der Wissenschaften zu Berlin, Institut für Ernährung, Potsdam-Rehbrücke

O. SELISKO und H. ACKERMANN

Eingegangen am 18. Februar 1963

[1]) SELISKO, O., u. H. ACKERMANN: Naturwissenschaften 46, 385 (1959). — [2]) SELISKO, O., G. HENTSCHEL u. H. ACKERMANN: Arch. intern. pharmacodynamie (im Druck). — [3]) GEBAUER, H., O. FUHRICH u. K. NICKISCH: Vitamine u. Hormone 7, 268 (1956). — [4]) KÄRBER, G.: Naunyn Schmiedeberg's Arch. exptl. Pathol. u. Pharmakol. 162, 480 (1931).

Zum Antagonismus Magnesium—Calcium

Im Zusammenhang mit der Bestimmung der LD_{50} verschiedener Mg-Salze (s. vorstehende Mitteilung) interessierte es uns, ob bei Verabfolgung von Magnesium und Calcium als Mischspritze die letale Dosis der *einzelnen* Präparate erreicht oder überschritten werden kann, ohne daß Exitus eintritt. Für diese Versuche wählten wir der Anionengleichheit wegen die Thiosulfate beider Metalle, und zwar die Handelspräparate „Magnosulf" (Nordmark-Werke) und „Calciumthiosulfat BC" (VEB Berlin-Chemie). Magnosulf enthält je ml 17,8 mg Magnesium, Calciumthiosulfat BC je ml 15,4 mg Calcium. Durch Mischung berechneter Volumina dieser wäßrigen Lösungen kann natürlich jedes gewünschte Mengenverhältnis Mg:Ca im Gemisch erhalten werden.

Es war zunächst erforderlich, für jedes der beiden Präparate die LD_{100}, also die kleinste Dosis, die alle Tiere einer Gruppe tötet, festzulegen. Unter den gleichen Versuchsbedingungen, wie in vorstehender Mitteilung beschrieben, fanden wir für LD_{100} bei Albinoratten:

Magnosulf: i.v. 18, i.p. 140 mg Mg/kg Gew.
Ca-Thiosulfat BC: i.v. 90, i.p. 150 mg Ca/kg Gew.

In zahlreichen Versuchen mit verschiedenen Mischungsverhältnissen beider Präparate stellten wir fest, daß die von allen Tieren tolerierte Dosis des Gemisches dann am höchsten lag, wenn der Mg-Gehalt der Lösung sich zum Ca-Gehalt wie 24:40 verhielt, also wie die Atomgewichte der beiden Metalle. Für *dieses* Gemisch (jedes andere ist weniger verträglich) geben wir folgende Werte der noch tolerierten und der bereits tödlichen Dosen an, und zwar wieder in Milligramm Metall/Kilogramm Körpergewicht:

	Alle Tiere tolerieren	LD_{100} des Gemisches
i. v.	32 mg Mg + 53 mg Ca (177%) + (60%)	42 mg Mg + 70 mg Ca (233%) + (77%)
i. p.	90 mg Mg + 150 mg Ca (64%) + (100%)	150 mg Mg + 250 mg Ca (107%) + (170%)

Die eingeklammerten Zahlen geben an, wieviel % der LD_{100} von Magnosulf bzw. Ca-Thiosulfat BC die angegebenen Mengen Mg und Ca ausmachen.

Es wäre möglich, daß der außerordentlich große antagonistische Effekt Magnesium—Calcium auch bei noch höheren Dosen wirksam wird, doch muß dann bereits eine schwere Hypermineralisation des Blutes vorhanden sein, die an sich zum Exitus führt.

Wir danken den Firmen Nordmark-Werke, Hamburg, und VEB Berlin-Chemie, Berlin-Adlershof, für die freundliche Überlassung von Präparaten.

Deutsche Akademie der Wissenschaften zu Berlin, Institut für Ernährung, Potsdam-Rehbrücke

O. SELISKO und H. ACKERMANN

Eingegangen am 18. Februar 1963

J Clin Invest, 39: 420-425 (1960)

MAGNESIUM TURNOVER IN THE HUMAN STUDIED WITH Mg^{28} *

By LAWRENCE SILVER, JAMES S. ROBERTSON AND LEWIS K. DAHL WITH THE TECHNICAL ASSISTANCE OF MARTHA HEINE AND LORRAINE TASSINARI

(From the Medical Research Center, Brookhaven National Laboratory, Upton, N. Y.)

(Submitted for publication September 1, 1959; accepted October 22, 1959)

Despite the relatively large amounts of magnesium present in the cells of most mammals, its *in vivo* functions in man remain poorly defined, a point which is emphasized in a recent review by Wacker and Vallee (1). There is evidence, which is still equivocal, that abnormalities of magnesium metabolism may be involved in several disease states including atherosclerosis (2), myxedema (3), hyperthyroidism (4), acute alcoholism (5), and possibly other conditions (6). Although Wacker and Vallee emphasized that, in view of its established capacity to function as an activator for many *in vitro* enzyme systems magnesium must play a significant role in intracellular metabolic activity, one of the intriguing aspects of magnesium has been its apparently relative inertness—particularly striking when compared with the other major ions in the body. Study of magnesium has been aided by the advent of the isotope Mg^{28} (7), although some caution has been necessary in using this isotope as well as in interpreting results obtained with it because of the relatively low specific activity which has been attained. With these limitations in mind we have made studies that tend to confirm earlier data arrived at by nonisotopic techniques, namely, that there are several relatively small, rapidly equilibrating compartments and one or more in which turnover is very slow. These data on man suggest that there are at least three compartments in the body pool of magnesium turning over with half-times of 1, 3, and 14 to 35 hours, respectively, but that probably 25 to 50 per cent of the magnesium has a turnover rate of less than 2 per cent per day.

MATERIALS AND METHODS

Clinical data. The 10 white adults—3 males of 45, 55 and 62 years of age, and 7 females aged 38, 46, 52, 53, 55, 64 and 72 years—were studied on the metabolic wards of the Hospital of the Brookhaven Medical Research Center for several months or more during the period of these investigations. All but the male aged 55 suffered from hypertension. Since the present observations are not clearly relevant to hypertension, only clinical data which might be pertinent to the present study are mentioned. The male of 55 had had classical rheumatoid arthritis of 3 years' duration but no other disease. The male of 62, who had a history of syphilis, was found to have positive serological tests for syphilis; the root of the ascending aorta was somewhat dilated by X-ray and a Grade 1 aortic diastolic murmur was present. In view of these findings, a diagnosis of luetic aortitis was made in addition to his undoubted essential hypertension of long standing. The remaining 8 patients had classical essential hypertension. In some patients cardiac enlargement was shown to be present by X-ray, and mild to moderate myocardial damage was evidenced by electrocardiographic changes; however, none had signs or symptoms, present or past, suggestive of heart failure. None of the patients gave a history of renal disease, and renal function studies including intravenous urograms were normal. All of the hypertensive subjects had negative Regitine tests for pheochromocytoma.

During the 8 day period, ranging from 5 days before to 3 days after administration of isotope, the patients were on a constant diet; although this was not identical from patient to patient, it was unvarying for any one patient, so that the magnesium content was fixed at about 50 to 60 mEq per day for each patient. All subjects had been on controlled dietary intakes previous to this study, with each diet calculated and weighed every day. Admission weight was maintained throughout hospitalization. Water intake was unrestricted although measured.

Methods. The isotope Mg^{28}, with a half-life of 21.3 hours, was obtained from the Brookhaven graphite reactor, and after appropriate purification and precipitation as $Mg^{28}(OH)_2$, was neutralized to pH 7.4 with HCl.[1] The highest specific activity that could be obtained ranged from 0.07 to 0.12 μc per mg of Mg^{++}, so that no carrier was added during administration or handling thereafter. After sterilization in an autoclave, the isotope was diluted with 5 per cent dextrose in water to a suitable volume, and delivered intravenously or orally to the patients from a calibrated 100 ml buret, in amounts ranging from 20 to 104 μc in total volumes ranging from 25 to 100 ml. The nonradioactive Mg^{++} infused intravenously ranged from 25 to 80 mEq and slow administration of the fluid was necessary to prevent toxic symptoms from the magnesium.

* This investigation was supported by the United States Atomic Energy Commission.

[1] The authors are indebted to Mr. H. O. Banks for the chemical preparation of the isotope.

flushing of the face was seen, and subjective feelings of heat in the face, feet, and lower abdominal and genital areas were complained of if the flow rate was too fast; no fall in blood pressure or change in reflexes occurred. Initial calculations indicated that the largest amount of isotope infused, assuming complete decay and no excretion, would give a maximal dose of slightly less than 0.3 rad whole-body irradiation. In light of the actual excretion data, the calculated radiation received may be decreased by approximately a quarter for the isotope administered intravenously.

Urine collections were made in chemically clean bottles; after the volume was measured, aliquots were removed, preserved with H_2SO_4 and stored at room temperature. Stools were similarly collected, homogenized with H_2SO_4, and an aliquot removed for digestion and hydrolysis according to the method of Wallace, Holliday, Cushman and Elkinton (8). Magnesium was measured with minor modifications by the micro-method of Karrman and Borgström (9), a method that was reproducible and accurate as indicated by the following data: *a*) standard solutions containing 10 μg Mg^{++} were titrated with each series of urine or plasma analyses and a random sampling of 40 such standards, extending over the course of these studies, gave a mean recovery value of 10.00 μg (SD ± 0.203); *b*) analysis of 10 replicates of a pooled plasma sample gave an average of 1.885 mEq per L. (SD ± 0.046), a value in good agreement with the results of others (9-11); *c*) to 3 series, each of 5 aliquots of this pooled plasma, the addition of 10, 20, and 40 μg Mg^{++} resulted in average recoveries of 99.1, 103.2 and 102.2 per cent, respectively. Radioactivity was measured with a well-type NaI scintillation crystal and standard scaler; duplicate 4 ml samples were counted sufficiently to make the counting error 1 per cent or less. In calculating the fraction of the total body Mg which had exchanged with the isotope, allowance was made for urinary or stool loss up to that time as well as for the Mg^{++} infused with the isotope.

RESULTS

Excretion of Mg^{++}

1. After oral administration. Two hypertensive subjects were given 80 μc Mg^{28} orally after which urine collections were made at intervals until the amount of radioactivity found was not significantly above background—about 60 hours later. All stools passed after the ingestion of the isotope were analyzed similarly. Plasma samples were counted at 2, 4, 15 and 39 hours after ingestion; these samples had maximal activity (corrected for decay) in the 15 hour samples. Because plasma activity was of such a low order in all samples—ranging from slightly over twice to a maximum of three times background—the curve of plasma activity has not been shown in Figure 1, which depicts only the urinary and stool excretion of the isotope. It is apparent that gastrointestinal absorption of magnesium was very limited in these two subjects, for in contrast to the results following intravenous administration described in the paragraph below, less than 3 per cent of the isotope was excreted in the urine in 60 hours, while between 10 and 20 times this fraction was found in the stools.

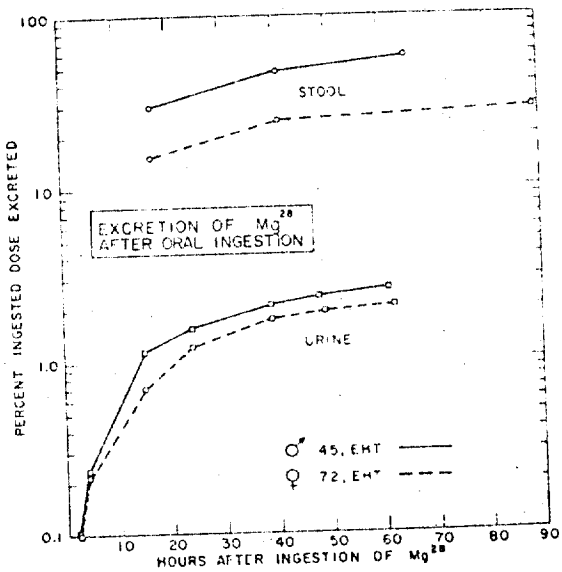


FIG. 1. STOOL AND URINE EXCRETION OF Mg^{++} AFTER ORAL ADMINISTRATION IN TWO PATIENTS WITH ESSENTIAL HYPERTENSION (EHT).

2. After intravenous administration. The data on nine subjects, including the nonhypertensive individual, are shown graphically in Figure 2. In contrast to the preceding study, excretion was virtually confined to the urine: 35 to 50 per cent of the radioactivity appeared in the urine before the isotope decayed to background levels, and in no instance did the stool account for more than about 1 per cent of the injected radioactivity (0.0 to 1.1 per cent). Approximately 25 per cent of the total radioactivity was found in the urines collected between two and five hours after injection of the isotope, values very similar to those found by Brandt, Glaser and Jones (12) for dogs after intravenous administration of this isotope.

The rapid urinary loss of the isotope after intravenous administration was mirrored in a rapid decline in counting rates of the plasma. The low concentration of magnesium in plasma in association with the relatively low specific activity of this

isotope combined to make data based on plasma counting unsuitable for accurate estimation of the time required for establishing equilibrium between tissues and isotope. Approximately 40 hours after intravenous administration the level of plasma activity was so low as to preclude further counting, and at this time equilibrium between tissues and isotope quite evidently had not yet been attained.

"Exchangeable" Mg

The concentration of Mg^{++} in the urine (2 to 10 × plasma) resulted in higher counting rates than was the case with plasma. The specific activities of the urinary Mg^{++} were used to calculate the "exchangeable" Mg^{++} data shown in Figure 3. Although in several instances the data suggest that exchange between isotopic and tissue Mg^{++} had reached a plateau level, in most instances this was not attained.

About 40 to 50 per cent of the injected dose of Mg^{28} was recovered in the urine during the first 48 hours after administration of the isotope, and only slightly more was recovered in two studies which could be continued for about 90 hours. Graphical analysis (13) of the urinary Mg^{28} curves in terms of exponential components (Figure 4) typically yielded a slow component of 14 to 38 hours half time, accounting for 10 to 15 per cent of the injected dose, and two more rapid components with one and three hour half-times, respectively, each accounting for 15 to 25 per cent of the injected dose. The sum of the quantities of Mg^{28} accounted for by integration of the components is about 10 per cent greater than the quantity actually recovered because of extrapolation beyond the last measurement. A few additional per cents of the administered dose were found in the stools. Even so, the total accounted for is typically only 50 to 75 per cent of the quantity injected. This indicates that there is at least one slow component not seen in the present data, and that 25 to 50 per cent of the magnesium in the body has a turnover rate of less than 2 per cent per day. It is important to recall that these turnover rates were arrived at in patients receiving magnesium both from the diet and from the low specific ac-

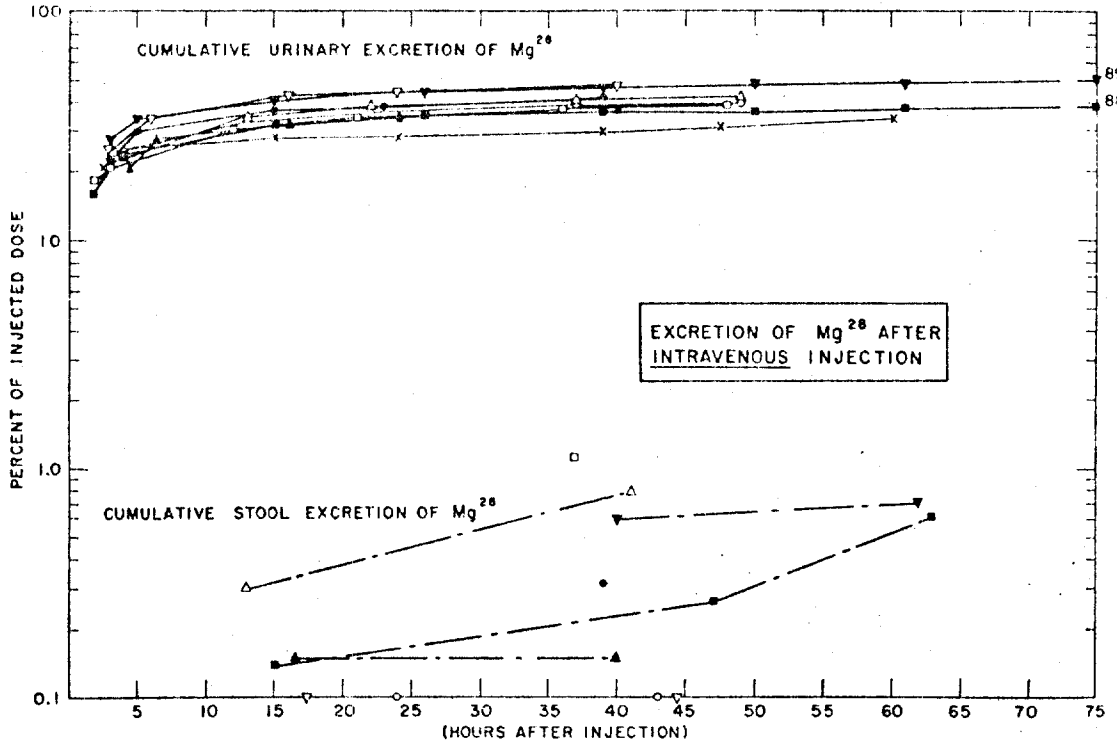


FIG. 2. STOOL AND URINE EXCRETION OF Mg^{28} AFTER INTRAVENOUS ADMINISTRATION IN NINE PATIENTS. Stools were not obtained on one patient.

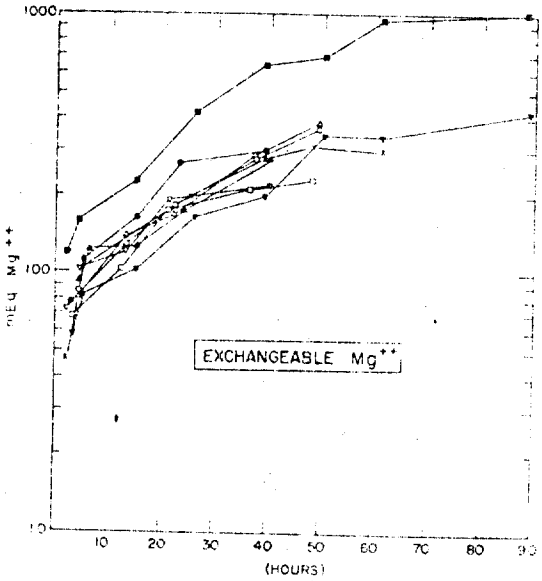


FIG. 3. CUMULATIVE ESTIMATE OF TOTAL EXCHANGEABLE MAGNESIUM AFTER INTRAVENOUS ADMINISTRATION OF Mg^{28} TO NINE PATIENTS.

tivity Mg^{28}. (The amount of magnesium injected was of the same order as that consumed on the constant diet in one day.) It is possible that the true turnover rates are much lower under conditions when lesser amounts of magnesium are being absorbed.

The data on Mg^{++} content of the human from whole body analysis are rather limited, but the estimates of Duckworth and Warnock (14), Forbes, Mitchell and Cooper (15) and Widdowson, McCance and Spray (16) are in rather good agreement, with values amounting to about 30 to 35 mEq per kg of body weight. Shohl's figures (17) are somewhat lower and it has been suggested that this was because of the limitation of the analytical method used for magnesium (16). In Table I are shown the values for exchangeable magnesium on the nine individuals in the present study; in only one of these patients was a value in excess of 10 mEq per kg obtained. It is of interest that five of the hypertensive subjects had values of 3.6 mEq per kg upon completion of the study, suggesting the interesting possibility that a biological invariant was being measured. These data may be interpreted to indicate that up to about 40 to 60 hours, perhaps 10 to 25 per cent of the tissue Mg had come into equilibrium with the isotope. The experiments which were extended for 88 and 89 hours in two individuals may have measured a third of the total body Mg^{++}.

DISCUSSION

Much of the early literature on the absorption and excretion of Mg was reviewed in Mendel and Benedict's paper (18) which appeared in 1909. Although there were considerable differences

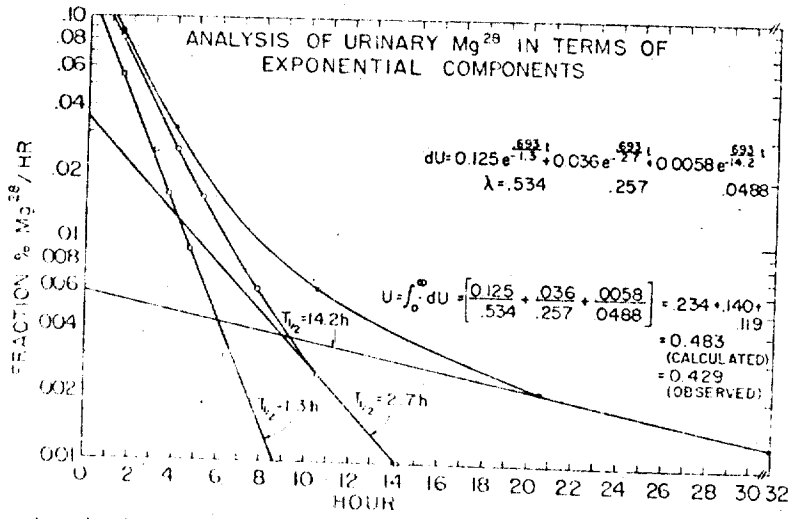


FIG. 4. ANALYSIS OF URINARY Mg^{28} EXCRETION IN TERMS OF EXPONENTIAL COMPONENTS (13). A. Fractional disappearance rates obtained from the expression $\lambda = 0.693/T_{1/2}$; U = amount of Mg^{28} excreted in the urine; dU = differential of Mg^{28} excretion in the urine.

TABLE I

Pertinent clinical data on nine subjects given Mg^{28} intravenously

Patient	Diagnosis	Age	Sex	Wt.	Time for equil. Equation	Exchangeable Mg^{++} Total	
		yrs.		*kg*	*hrs.*	*mEq*	*mEq/kg*
C	EHT*	46	♀	82.6	39	300	3.6
Bl	EHT	72	♂	61.7	40	220	3.6
K	EHT	52	♀	76.0	40	277	3.6
W	EHT	53	♂	64.2	48	232	3.6
H	RA	55	♂	73.1	49	365	5.0
S	EHT	64	♀	55.9	49	380	6.8
L	EHT	55	♀	83.5	61	304	3.6
Bi	EHT and LHD	62	♂	83.2	88	1,040	14.1
D	EHT	38	♀	46.4	89	431	9.3

* EHT = essential hypertension; RA = rheumatoid arthritis; LHD = luetic heart disease.

among the early data and the interpretation thereof, Mendel and Benedict showed quite clearly that the subcutaneous injection of various Mg^{++} salts was followed by rapid urinary excretion of the ion, whereas intestinal excretion, if present at all, was insignificant. Hirschfelder and Haury (19), however, reported that in seven normal adults 40 to 44 per cent of an ingested dose of Mg^{++} appeared in the urine within 24 hours. Tibbets and Aub (20) by means of classical balance techniques studied the excretion of Mg^{++} in normal subjects; they found that individuals on an intake of 0.6 to 0.9 g per day excreted 0.5 to 0.8 g, of which slightly over half was in the stools. Smith, Winkler and Schwartz (21) studied the excretion of Mg^{++} in dogs after intravenous administration of $MgSO_4$ and concluded that during the first three to four hours the Mg^{++} distributed itself throughout the extracellular fluid, while during subsequent hours there was evidence that some of the ion was being segregated from the extracellular fluid and not excreted. Both the isotopic studies by Rogers and Mahan on rats (22) and the current study on humans are in general agreement with this earlier nonisotopic work. Rogers and Mahan concluded that in rats magnesium was present in two or more physiologic states, one with a turnover time of 1.2 hours, and the other with about 25 hours. As noted earlier, our data suggest two rapidly equilibrating pools with half-times of 1 and 3 hours, a slower one of 14 to 35 hours, and one (or more) with a very slow turnover of the order of 2 per cent per day or less.

In the present work, intestinal absorption of Mg^{++} was low, but the data were derived from single experiments in two individuals. It is possible that lack of absorption was due to the formation of relatively insoluble Mg^{++} compounds in the alkaline medium of the intestine or that absorption is dependent upon body need. The fact that only about 1 per cent of an intravenous load of Mg^{28} appeared in the stools is strongly against the possibility that magnesium is absorbed from the gut at one point and re-excreted into it at another. The marked disparity between these data and some of those derived from nonisotopic studies (19) indicates the need for further study with the isotope, possibly using several other salts of Mg^{++}. However, Aikawa, Rhoades and Gordon (23) using $Mg^{28}Cl$ in man, and Aikawa (24) using $Mg^{28}SO_4$ in rabbits, also found low urinary excretion after oral administration of the isotope.

If approximately three-fourths of the total body Mg^{++} is assigned to bone (14, 15) and the assumption made that none of this is exchangeable, the present data indicate that perhaps half of the remaining Mg^{++} has exchanged with the isotope 40 hours after intravenous administration. It is more likely that some of the bone Mg is exchangeable (12). Although the exact size of this fraction may be in question, it is clear that somewhere there is a relatively large pool with a slow turnover rate and presumably this is made up largely by the magnesium in bone. Based on the analyses previously cited (14–16) only about 10 to 35 per cent of the total Mg^{++} stores can be assumed to have exchanged with the isotope after 40 to 90 hours of equilibration.

SUMMARY

Studies with Mg^{28} in man suggest that gastrointestinal absorption of Mg^{++} is very limited. Fol-

lowing intravenous administration the isotope rapidly appears in the urine but only insignificant amounts appear in the stool. This indicates that most of the magnesium in the stools is of exogenous origin. Equilibration of the isotope with magnesium in the body is slow and at 40 to 60 hours it amounts to about 10 to 25 per cent of the total; at 90 hours perhaps a third of the body's magnesium has reached equilibrium with the isotope. Graphical analysis of urinary Mg^{28} curves in terms of exponential components yielded a slow component with a half-time of 14 to 35 hours accounting for 10 to 15 per cent of the injected dose, and two more rapid components with half-times of 1 and 3 hours each, accounting for 15 to 25 per cent of the injected dose. The large fraction remaining—from about 25 to 50 per cent of the body's total—has a turnover rate of less than 2 per cent per day. These turnover values must be interpreted with caution because of possible effects from the significant amounts of dietary magnesium in addition to the effect of that administered with the low specific activity Mg^{28}.

ACKNOWLEDGMENTS

We wish to thank the Misses Josephine O'Connell, R. N., Mary Craig, R. N., and Norma Gillespie, dietitian, for their cooperation and assistance with these studies.

REFERENCES

1. Wacker, W. E. C., and Vallee, B. L. Magnesium metabolism. New Engl. J. Med. 1958, **259**, 431 and 475.
2. Jankelson, O. M., Vitale, J. J., and Hegsted, D. M. Serum magnesium, cholesterol, and lipoproteins in patients with atherosclerosis and alcoholism. Amer. J. clin. Nutr. 1959, **7**, 23.
3. Tapley, D. F. Magnesium balance in myxedematous patients treated with triiodothyronine. Johns Hopk. Hosp. Bull. 1955, **96**, 274.
4. Soffer, L. J., Cohn, C., Grossman, M. I., and Sobotka, H. Magnesium partition studies in Graves' disease and in clinical and experimental hypothyroidism. J. clin. Invest. 1941, **20**, 429.
5. Blink, E. B., Stutzman, F. L., Anderson, A. R., Konig, T., and Frasch, R. Magnesium deficiency after prolonged parenteral fluid administration and after chronic alcoholism complicated by delirium tremens. J. Lab. clin. Med. 1954, **43**, 169.
6. Haury, V. G., and Cantarow, A. Variations of serum magnesium in 52 normal and 440 pathologic patients. J. Lab. clin. Med. 1942, **27**, 616.
7. Sheline, R. K., and Johnson, N. R. Decay scheme of magnesium 28. Phys. Rev. 1953, **90**, 325.
8. Wallace, W. M., Holliday, M., Cushman, M., and Elkinton, J. R. The application of the internal standard flame photometer to the analysis of biologic material. J. Lab. clin. Med. 1951, **37**, 621.
9. Karrman, K. J., and Borgström, S. An accurate micromethod for the estimation of magnesium in biological material, particularly blood serum. Svensk kem. T. 1955, **67**, 18.
10. Hunter, G. Calcium and magnesium content of normal human blood serum. Nature (Lond.) 1958, **182**, 263.
11. Robinson, R. R., Murdaugh, H. V., Jr., and Peschel, E. Renal factors responsible for the hypermagnesemia of renal disease. J. Lab. clin. Med. 1959, **53**, 572.
12. Brandt, J. L., Glaser, W., and Jones, A. Soft tissue distribution and plasma disappearance of intravenously administered isotopic magnesium with observations on uptake in bone. Metabolism 1958, **7**, 355.
13. Robertson, J. S. Theory and use of tracers in determining transfer rates in biological systems. Physiol. Rev. 1957, **37**, 133.
14. Duckworth, J., and Warnock, G. M. Magnesium requirements of man in relation to calcium requirements, with observations on adequacy of diets in common use. Nutr. Abstr. Rev. 1942, **12**, 167.
15. Forbes, R. M., Mitchell, H. H., and Cooper, A. R. Further studies on the gross composition and mineral elements of the adult human body. J. biol. Chem. 1956, **223**, 969.
16. Widdowson, E. M., McCance, R. A., and Spray, C. M. The chemical composition of the human body. Clin. Sci. 1951, **10**, 113.
17. Sholl, A. T. Mineral Metabolism. New York, Reinhold, 1939, p. 384.
18. Mendel, L. B., and Benedict, S. R. The paths of excretion for inorganic compounds. IV. The excretion of magnesium. Amer. J. Physiol. 1909, **25**, 1.
19. Hirschfelder, A. D., and Haury, V. G. Clinical manifestations of high and low plasma magnesium; dangers of epsom salt purgation in nephritis. J. Amer. med. Ass. 1934, **102**, 1138.
20. Tibbets, D. M., and Aub, J. C. Magnesium metabolism in health and disease. I. The magnesium and calcium excretion of normal individuals, also the effects of magnesium, chloride and phosphate ions. J. clin. Invest. 1937, **16**, 491.
21. Smith, P. K., Winkler, A. W., and Schwartz, B. M. The distribution of magnesium following the parenteral administration of magnesium sulfate. J. biol. Chem. 1939, **129**, 51.
22. Rogers, T. A., and Mahan, P. E. Exchange of radioactive magnesium in the rat. Proc. Soc. exp. Biol. (N. Y.) 1959, **100**, 235.
23. Aikawa, J. K., Rhoades, E. L., and Gordon, G. S. Urinary and fecal excretion of orally administered Mg^{28}. Proc. Soc. exp. Biol. (N. Y.) 1958, **98**, 29.
24. Aikawa, J. K. Gastrointestinal absorption of Mg^{28} in rabbits. Proc. Soc. exp. Biol. (N. Y.) 1959, **100**, 293.

J. Biol. Chem., 129: 51-56, 1939

THE DISTRIBUTION OF MAGNESIUM FOLLOWING THE PARENTERAL ADMINISTRATION OF MAGNESIUM SULFATE*

By PAUL K. SMITH, ALEXANDER W. WINKLER,† AND BERNARD M. SCHWARTZ‡

(From the Departments of Pharmacology and Toxicology and of Internal Medicine, Yale University School of Medicine, New Haven)

(Received for publication, April 1, 1939)

There is reason to believe that sodium, chloride, bromide, sulfate, and thiocyanate ions when injected into the body are distributed through the extracellular fluid alone (2, 5-7, 9, 10). Potassium on the other hand is distributed through a much larger proportion of the body water (11). Thus, of the two cations thus far studied, one, sodium, is excluded from cells, while the other, potassium, appears to enter cells with some freedom. In order to observe the behavior of another naturally occurring cation the distribution of magnesium after injection has been studied and is the subject of the present report.

The general method of study is similar to that previously applied to a study of potassium distribution, and discussed in detail in a previous communication (11). Isotonic magnesium sulfate solution was injected intravenously into dogs, and serum and urine collections made at intervals following injection. By the use of the formula

$$\text{Apparent volume of distribution} = \frac{\text{amount injected minus amount excreted in urine}}{\text{increase in concentration in serum water}}$$

* A preliminary report was presented before the American Society of Biological Chemists at the annual meeting at Toronto, Canada, April 27-29, 1939 (Winkler, A. W., Smith, P. K., and Schwartz, B. M., *Proc. Am. Soc. Biol. Chem.*, *J. Biol. Chem.*, **128**, p. cxii (1939)).

† Aided by a grant from the Ella Sachs Plotz Fund.

‡ From a thesis submitted by Bernard M. Schwartz to the faculty of the Yale University School of Medicine in candidacy for the degree of Doctor of Medicine, 1939.

independent calculations of the volumes of distribution of magnesium and of sulfate were made. By a comparison of these figures it could be determined whether magnesium behaved as did sulfate, and, assuming a purely extracellular distribution of sulfate, whether or not it remained confined to the extracellular fluid.

In another group of experiments the problem was approached in a somewhat different manner. Following the injection intraperitoneally of magnesium sulfate all urine and feces were collected during the next 24 hours. The concentration of magnesium in the serum at the end of the period was compared with the concentration preceding injection. From these data the retention of any magnesium in the body could be determined, and the amount of magnesium still left in the extracellular fluid could be calculated. If some of the magnesium retained could not be accounted for in the extracellular fluid, then of necessity it must have been deposited elsewhere.

Materials and Methods

Adult, unanesthetized female dogs were used. In some a preliminary dose of morphine (10 mg. per kilo) was administered. Urine specimens were obtained by means of a retention catheter. Injections were made into the femoral vein and blood specimens obtained from either the femoral or the jugular vein. Isotonic magnesium sulfate solution (0.154 M) was used in all experiments, the rate of injection approximating 10 cc. per minute. Injections were terminated before the appearance of respiratory arrest. In the stool recovery experiments carmine was given by mouth at the time of injection and all stools saved until they became carmine-free.

Magnesium was determined in serum, urine, and stools by a modification of the method of Hald (4). The calcium was first removed by precipitation as the oxalate at pH 4.2, 0.2 N acetate buffer being used. The magnesium was precipitated as magnesium ammonium phosphate, some hours being allowed for complete precipitation and care being taken to preserve a constant temperature during the process. Finally the magnesium was determined colorimetrically as phosphate by the method of Fiske and Subbarow (3). Sulfate was determined in serum and in urine by the method of Cope (1). Concentrations in terms of

serum water were obtained by dividing determined serum concentrations by 0.92.

Results

In Table I are summarized the results of five experiments with four dogs, in which the volume of distribution was determined

TABLE I

Distribution of Magnesium and Sulfate Following Intravenous Injection of Magnesium Sulfate

Experiment No.	Dog	Dog weight	$MgSO_4$ given	Time after administration	Mg		SO_4		Volume of distribution		Volume of distribution	
					Increase in serum water	Remaining in body	Increase in serum water	Remaining in body	Mg	SO_4	Mg	SO_4
		kg.	*m.eq.*	*min.*	*m.eq. per l.*	*m.eq.*	*m.eq. per l.*	*m.eq.*	*liters*	*liters*	*per cent body weight*	*per cent body weight*
1	A	17.4	55.4	9	14.5	45.0	12.7	40.9	3.1	3.5	18	20
				42	7.9	35.9	6.7	28.3	4.5	4.2	25	24
				78	5.3	29.0	3.9	18.8	5.5	4.8	31	27
2	"	18.9	50.5	12	11.6	40.9	11.2	39.4	3.5	3.5	19	19
				79	7.2	30.1	5.7	24.5	4.2	4.3	22	23
				157	5.0	23.9	3.3	17.4	4.8	5.3	25	28
				225	3.5	14.3	2.1	11.9	5.5	5.7	29	30
3	B	18.8	49.6	16	13.9	47.3	13.5	46.3	3.4	3.4	18	18
				56	10.5	37.3	8.2	32.1	3.5	3.9	19	21
				116	5.0	26.7	4.6	17.7	5.3	3.9	29	21
4	C	11.7	36.0	6	13.6	30.5	11.3	28.6	2.2	2.5	19	21
				77	6.7	19.8	4.0	15.1	3.0	3.8	26	32
				148	4.0	14.9	1.6	8.7	3.7	*	32	*
5	D	30.3	77.0	8	12.9	68.6	10.9	62.3	5.3	5.7	18	19
				56	8.5	57.7	6.4	44.2	6.8	6.9	22	23
				156	6.2	44.1	2.9	20.8	7.1	7.2	23	24

* The increment of sulfate in serum was too small for accurate calculation.

after intravenous injection. The apparent volumes of distribution of magnesium are in general identical with those of sulfate. The only possible exceptions occur in the third period of Experiment 3, in which that of magnesium is somewhat higher, and in the second period of Experiment 4, in which it is somewhat lower. There is a tendency in all experiments for the apparent volume of

distribution of both ions to rise in later periods. No experiments were continued beyond 3 or 4 hours, since by that time the concentration increments of both ions in the serum had fallen to nearly to normal levels for accurate calculations to be made. The actual volumes of distribution corresponded to 18 to 32 per cent

TABLE II

Recovery of Magnesium in Urine 24 Hours after Intravenous Injection

Experiment No.	$MgSO_4$ given	Recovered in urine within 24 hrs.	Unaccounted for in urine	Increase in concentration in serum water at end of 24 hrs.	Extracellular fluid volume	Accounted for in extracellular fluid	Unaccounted for in urine or extracellular fluid	
	m.eq.	*m.eq.*	*m.eq.*	*m.eq. per l.*	*liters*	*m.eq.*	*m.eq.*	*per cent*
1	55.4	27.6	27.8					
2	50.5	43.6	6.9	0.3	5	1.5	5.4	11
3	49.6	27.6	26.7	0.1	5	0.5	26.2	53
4	36.0	26.4	11.0	2.3	3	6.9	4.1	16
5	77.0	52.3	24.7	1.8	7	12.6	12.1	16

TABLE III

Recovery of Magnesium in Urine and Feces in 24 Hours after Intraperitoneal Injection

Experiment No.	$MgSO_4$ given	Recovered in urine in 24 hrs.	Recovered in feces in 24 hrs. or more	Increase in concentration in serum water at end of 24 hrs.	Extracellular fluid volume	Accounted for in extracellular fluid	Unaccounted for		SO_4 recovered from urine alone during same period	
	m.eq.	*m.eq.*	*m.eq.*	*m.eq. per l.*	*liters*	*m.eq.*	*m.eq.*	*per cent*	*m.eq.*	*per cent*
6	77.0	18.0	10.5	1.8	4	7.2	41.3	54	81.8	109
7	46.2	23.6	3.3	0.0	4	0.0	19.5	42	50.2	114
8	46.2	39.7	2.3	0.0	3	0.0	4.2	9	42.9	95
9	77.0	40.3	19.9*	0.4	4	1.6	15.8	21	75.5	104

* Includes stools for 4 days after injection.

of the body weight, a range consistent with the magnitude of the extracellular fluid.

In Table II are summarized the total amounts of magnesium recovered from the urine in the 24 hours after injection in the five experiments of Table I. Initial and final determinations of

the concentrations in the serum are included. Even without allowance for endogenous magnesium excretion during the 24 hour period, failure to recover the injected magnesium in the urine is evident. The magnitude of the changes in serum concentration indicates that this retention can only in part be accounted for by the amount of magnesium still present in the extracellular fluid.

In Table III are presented four complete recovery experiments on four dogs. The magnesium sulfate was given intraperitoneally. In three of the four experiments the same marked retention of magnesium is present. The stool excretion figures are if anything too high, since they include the magnesium already present; in spite of this the amounts are small and in no way account for the failure of recovery in the urine. Therefore, in three of the four experiments magnesium in significant quantities is clearly retained within the body, but not in the extracellular fluid. Simultaneously injected sulfate was completely recovered from the urine within 24 hours.

DISCUSSION

The nearly identical volumes of distribution of magnesium and of sulfate in the first 3 or 4 hours after injection indicate that both ions are distributed throughout the same portion of the body water. The magnitude of this portion, corresponding to about one-fourth the body weight, indicates that it is reasonable to identify it with the extracellular fluid volume. Thus magnesium resembles sodium rather than potassium in its initial distribution after injection, since sodium and sulfate distributions are virtually identical. The apparent gradual increase in the volume of distribution of the ions as time elapses is probably due to concentration gradients, first from serum to extracellular fluid and then in the reverse direction. In other words the earlier calculated volumes of distribution are probably lower, the later higher, than the true volume of distribution.

The failure in most experiments to recover all the injected magnesium in stools and urine within 24 hours is probably even more marked than the figures in Tables II and III indicate, since the stool and urine values include endogenous magnesium and so represent maxima for the period. Indeed there is no evidence that any of the injected magnesium is excreted by way of the

feces, since the figures for 24 hour loss in stools after injection fall within the limits of normal daily fecal excretion. This failure of recovery is a confirmation of the work of Mendel and Benedict (8), who, after injection of magnesium salts, were unable to recover the magnesium completely for days or weeks. Since it cannot be accounted for in the extracellular fluid, the conclusion seems inescapable that sometime between the 4th and the 24th hour after injection some of the magnesium may leave extracellular fluid to be segregated elsewhere. There is no evidence from these experiments as to the mode or the place of this deposition.

SUMMARY

1. The magnesium of magnesium sulfate injected intravenously into dogs distributes itself immediately through approximately the same volume of fluid as does the sulfate ion, corresponding to some 20 or 25 per cent of the body weight.

2. This volume is believed to approximate the extracellular fluid of the body, so that magnesium behaves in respect to its distribution like sodium rather than potassium.

3. Between 4 and 24 hours after injection a variable proportion of the magnesium injected, over and above what can be accounted for in urine and stools, may leave the extracellular fluid and be segregated in unknown form elsewhere in the body.

BIBLIOGRAPHY

1. Cope, C. L., *Biochem. J.*, **25**, 1183 (1931).
2. Crandall, L. A., Jr., and Anderson, M. X., *Am. J. Digest. Dis. and Nutrition*, **1**, 126 (1934).
3. Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, **66**, 375 (1925).
4. Hald, P. M., *J. Biol. Chem.*, **103**, 471 (1933).
5. Harrison, H. E., Darrow, D. C., and Yannet, H., *J. Biol. Chem.*, **113**, 515 (1936).
6. Hastings, A. B., and Eichelberger, L., *J. Biol. Chem.*, **117**, 73 (1937).
7. Lavietes, P. H., Bourdillon, J., and Klinghoffer, K. A., *J. Clin. Inv.*, **15**, 261 (1936).
8. Mendel, L. B., and Benedict, S. R., *Am. J. Physiol.*, **25**, 1 (1909).
9. Smith, P. K., and Walker, D. W., *J. Pharmacol. and Exp. Therap.*, **63**, 35 (1938).
10. Wallace, G. B., and Brodie, B. B., *J. Pharmacol. and Exp. Therap.*, **61**, 397 (1937).
11. Winkler, A. W., and Smith, P. K., *J. Biol. Chem.*, **124**, 589 (1938).

Arch. Neurol. & Psychiat. 63:749-759 (1950)

MAGNESIUM INTOXICATION

Absorption from the Intact Gastrointestinal Tract

ALEXANDER R. STEVENS Jr., M.D.
AND
HAROLD G. WOLFF, M.D.
NEW YORK

MAGNESIUM sulfate U. S. P. (Epsom salt) is used in medicine chiefly for two effects. When given parenterally in small doses, it is an effective cerebral depressant, and therefore useful in the control of convulsions. When introduced into the gastrointestinal tract in hypertonic solution it is considered to be poorly absorbed, and therefore useful for its osmotic effect. In drawing water from the body, it accomplishes both dehydration and diarrhea. In amounts of 15 to 20 Gm. it is often given by mouth as a laxative, and in approximately the same amounts it is one of the constituents of the familiar "magnesium sulfate, glycerin and water" enema.

This report is chiefly concerned with the widespread practice of administering large amounts of magnesium sulfate by slow drip per rectum to induce dehydration in the management of increased intracranial pressure and cerebral edema. It has usually been assumed that this procedure is harmless because magnesium sulfate is poorly absorbed from the rectum, and that therefore it is a useful preoperative procedure for patients with brain tumor and increased intracranial pressure.[1] Recent experience with 2 patients has led to a reevaluation of the pharmacology of magnesium sulfate.

In 1909 Fraser[2] reviewed the literature from 1841 to 1909 on the toxicity of Epsom salt and found evidence of poisoning in 7 cases, with 5 fatalities. All the patients had taken the salt by mouth, and depression of the central nervous system was the most serious toxic effect. Fraser suggested that the toxicity might be based on a central effect, from

From the New York Hospital and the Departments of Medicine (Neurology) and Psychiatry, Cornell University Medical College.

1. Fay, T.: The Administration of Hypertonic Salt Solutions for the Relief of Intracranial Pressure, J. A. M. A. **80**:1445 (May 19) 1923; Comparative Values of Magnesium Sulphate and Sodium Chloride for Relief of Intracranial Tension, ibid. **82**:766 (March 8) 1924.

2. Fraser, C.: Epsom Salts as a Poison, with a Record of a Case of Unusual Symptoms Due to This Drug, Lancet **1**:1174, 1909.

absorption of the drug. In the same year Mendel and Benedict[3] reported on the excretion of magnesium salts. When the salts were given parenterally to animals, 80 per cent could be recovered from the urine within forty-eight hours. The role of the kidneys in excreting magnesium salts has been repeatedly emphasized, and in 1934 Hirschfelder[4] pointed out that in persons with renal damage oral administration of magnesium sulfate may be associated with elevation of the magnesium level of the plasma from a normal of 2 mg. to as high as 8 or 15 mg. per hundred cubic centimeters, with accompanying drowsiness and near coma. The same investigator produced renal damage in rabbits, and in these animals the administration of magnesium sulfate by mouth led to high magnesium levels in the blood. Values of 17 mg. per hundred cubic centimeters or higher were associated with coma in the rabbits. It was pointed out that magnesium coma may be difficult to distinguish from uremic coma. Hirschfelder also gave 20 to 30 Gm. of magnesium sulfate by mouth to persons with normal renal function and obtained "insignificant" increases of the plasma magnesium (0.2 to 0.4 mg. per hundred cubic centimeters), but he recovered 40 to 44 per cent of the ingested magnesium in the urine within twenty-four hours, thus demonstrating that a large amount had been absorbed. The magnesium content of the stools was not measured.

Hoff,[5] in 1939, studied the toxic effects of magnesium sulfate in dogs. In isotonic solution, the salt was injected intravenously, and levels of serum magnesium were correlated with observations on blood pressure, respiration and general behavior. A decline in blood pressure was noted with a level of 6 mg. per hundred cubic centimeters, or even below, and thereafter evidence of increasing cerebral depression. A level of 18 to 20 mg. per hundred cubic centimeters was associated with respiratory arrest, and when the dogs were kept alive with artificial respiration, a level of 32 to 46 mg., with cardiac arrest. Neuwirth and Wallace,[6] exploring the possibilities of magnesium as an aid in anesthesia, reported that in healthy dogs a serum magnesium level of 5 mg. per hundred cubic centimeters was correlated with definite evidence of cerebral depression, and one of 19 to 23 mg. per hundred cubic centimeters, with complete anesthesia. These investigators also reported that the rectal administra-

3. Mendel, L. B., and Benedict, S. R.: The Paths of Excretion for Inorganic Compounds: IV. The Excretion of Magnesium, Am. J. Physiol. **25**:1, 1909.

4. Hirschfelder, A. D.: Clinical Manifestations of High and Low Plasma Magnesium: Dangers of Epsom Salt Purgation in Nephritis, J. A. M. A. **102**:1138 (April 7) 1934.

5. Hoff, H. E.; Winkler, A. H., and Smith, P. K.: Cardiovascular and Neuromuscular Changes Following Intravenous Injections of Magnesium Salts, Am. J. Physiol. **126**:537, 1939.

6. Neuwirth, I., and Wallace, G. B.: On Use of Magnesium as an Aid in Anesthesia, J. Pharmacol. & Exper. Therap. **35**:171 (Feb.) 1929.

tion of 14 Gm. of magnesium sulfate to healthy dogs, and of 8 Gm. to healthy human subjects, resulted in insignificant elevation of the serum magnesium. They did not give larger amounts by rectum.

In 1922 Rector,[7] also interested in the potentialities of magnesium sulfate in anesthesia, gave to each of 5 patients 210 cc. of a 25 per cent solution by rectum. In all he obtained significant elevations of the serum magnesium, the average value at one hour being about 10 mg. per hundred cubic centimeters and the level falling to normal in four hours. Unfortunately, no clinical observations were reported.

In 1943 Fawcett and Gens[8] described 2 cases in which signs of magnesium poisoning followed an enema of Epsom salt. In the first, that of an infant, the diagnosis was made on clinical signs, no samples of blood being studied. In the second, a woman aged 23 with a ureteral stone, who was otherwise healthy, and presumably had normal renal function, was given an enema containing about 25 Gm. of magnesium sulfate with glycerin; this was assumed to be retained in the rectum approximately two minutes. After thirty minutes she felt hot and thirsty. One hour and fifteen minutes after the enema, she was found unresponsive, with respiration entirely abdominal and with complete areflexia. Intravenous injection of calcium, the pharmacologic antidote for magnesium, was given, with a dramatic response, the patient regaining consciousness within a few minutes. Three hours after the enema had been administered, blood was drawn, and its serum magnesium value was 20.8 mg. per hundred cubic centimeters. This is the highest value we have found reported in a human subject. Fawcett and Gens concluded that the intact bowel of some persons exhibits a peculiar ability to absorb magnesium sulfate.

At the New York Hospital, one-half saturated solution of magnesium sulfate is often given to patients with increased intracranial pressure, in the amount of 300 cc. administered by slow Murphy drip per rectum, at a rate of 1 drop per second, so that the whole volume is administered over a period of three hours or longer. Three hundred cubic centimeters of such a solution contains 94.5 Gm. of magnesium sulfate U. S. P. At the beginning of the enema, the patient may be nearly comatose from the effects of the increased intracranial pressure, and, as the dehydrating effect of the procedure becomes manifest, the patient characteristically becomes more alert. Significant absorption of the salt from the rectum has not until recently been considered a dangerous possibility. However, that untoward sequelae may occur when high

7. Rector, J. M.: Synergistic Analgesia: Clinical Observations, Am. J. Surg. (Anesthesia supp.) **36**:114 (Oct.) 1922.

8. Fawcett, D. W., and Gens, J. P.: Magnesium Poisoning Following an Enema of Epsom Salt Solution, J. A. M. A. **123**:1028 (Dec. 18) 1943.

concentrations are used is demonstrated by the 2 protocols which follow. To each of the patients, a fully saturated solution of magnesium sulfate was given for the purpose of accelerating dehydration.

REPORT OF CASES

CASE 1.—A white girl aged 18 was admitted to New York Hospital for the fourth time because of repeated convulsions of the left side. During her first admission, four and one-half years earlier, an unclassified glioma was removed from the right temporoparietal area, and after operation the tumor site was exposed to repeated roentgen treatments. Subsequent admissions were for the control of convulsions involving the left side, which usually diminished in number after large amounts of phenobarbital were administered. For about two months before her final admission, the patient had increased considerably her fluid intake, chiefly with milk and cola beverages. She became irregular in taking her prescribed doses of phenobarbital. Two weeks prior to admission numbness of the left hand and right-sided headache were noted. The day before admission she began to have convulsions of the left side, which continued despite anticonvulsant medication. Physical examination revealed no definite change from that described on her previous admission, two years earlier. The findings included a bony defect in the right temporoparietal region, normal eyegrounds, weakness of the left side, pathologic reflexes and absence of the abdominal reflexes. The patient had frequent convulsions on the left side and was regarded as being in status epilepticus. At first she was given sodium phenobarbital subcutaneously, 0.13 Gm. after each convulsion, but this was reduced to 0.065 Gm. on the second day. The frequency of seizures was diminishing until the third night in the hospital, when she had several of long duration. Because of the history of increased fluid intake preceding the present series of seizures, it was considered that cerebral edema might be a factor. It was decided therefore to reduce the edema by a dehydrating procedure. Accordingly, 300 cc. of saturated solution of magnesium sulfate (189 Gm. of the salt) was administered per rectum, by slow Murphy drip, over a period of three hours. At the beginning of administration of the enema, the patient was listless, a state which was attributed to the barbiturates given during the preceding three days. One hour later the patient became restless and complained of local rectal irritation. At two and one-half hours her nurse noted that she was "very drowsy, but restless at intervals." At three hours, just as the enema was completed, the patient turned herself over onto her abdomen. Ten minutes later it was noted that she was cyanotic, with shallow respirations. The patient was turned on her back, and artificial respiration was begun immediately, resulting in a decided improvement in color. Although the heart beat was not audible through the chest wall at this time, a weak radial pulse was felt. Caffeine and sodium benzoate U. S. P., 0.5 Gm., was given intramuscularly, without effect. After ten minutes of manual artificial respiration, pure oxygen was given by intratracheal tube, with use of intermittent positive pressure. The radial pulse was no longer palpable, and the patient was pronounced dead within one-half hour of the termination of the magnesium sulfate enema.

Postmortem examination revealed extensive cerebral atrophy and a surgical defect in the right cerebral cortex. Microscopic examination showed no evidence of tumor, and the changes were gliosis and vascular thickening in the area exposed to roentgen radiation. A review of the microscopic sections from the original tumor, removed at operation, led to the diagnosis either of neurospongioblastoma

or of glioblastoma multiforme. The kidneys were entirely normal, grossly and microscopically, and it is of importance that during life, the results of urinalyses were unremarkable. No formal studies of renal function had ever been made.

Within one hour post mortem, specimens of heart's blood and spinal fluid were obtained. Magnesium levels were determined according to the method of Denis.[9] The value for the serum was found to be 20.1 mg. per hundred cubic centimeters and that for the spinal fluid, 3.1 mg., per hundred cubic centimeters. The latter is normal, but the serum level corresponds to the toxic levels reported in the literature.

CASE 2.—A white woman aged 42 was admitted for the second time to New York Hospital because of numbness, weakness and convulsive movements of the left arm and leg. On her previous admission, sixteen months earlier, a glioblastoma multiforme had been partially resected from the right frontoparietal area. On admission, she was mentally alert, but exhibited weakness and hyperactive reflexes on the left side of the body and bilateral papilledema. In preparation for head surgery, a slow drip of magnesium sulfate solution per rectum was administered. After 100 cc. had been given, the patient was flushed and drowsy. After 200 cc., she was extremely lethargic, responding only to repeated noxious stimulation. After 250 cc., and at the end of three hours, she was comatose, although the vital signs were unchanged; and the drip was discontinued. All the fluid had been retained, and difficulty was experienced in obtaining returns, even after several enemas of soapsuds. Fifty cubic centimeters of 50 per cent dextrose was given intravenously, with slight immediate effect. The patient gradually became less drowsy and returned to full consciousness about two or three hours after the magnesium sulfate enema had been discontinued. No samples of blood were obtained during the episode. Because this was the only loss of consciousness which the patient experienced during this phase of her illness, it seemed logical to attribute it to the cerebral depressant effect of magnesium absorbed from the rectum. Studies of the urine demonstrated normal renal function, including adequate concentration.

EXPERIMENTAL STUDY

SERIES 1.—In order to learn more about the rectal absorption of magnesium sulfate, and to correlate more accurately the serum level of magnesium with the clinical state of the subject, the following experiment was performed. Voluntary participation was obtained of 6 male subjects, all of whom had normal urine and none of whom was taking digitalis. None of the subjects was receiving sedative medication. All received hypertonic solution of magnesium sulfate per rectum by Murphy drip, at a rate of approximately 1 drop per second, and it was planned to give 300 cc. to each. Subjects A, B, C and D received a one-half saturated solution, and subjects E and F received a saturated solution. All were carefully observed for evidence of drowsiness and were repeatedly asked to report on sensations of heat or thirst. Blood was withdrawn at approximately hourly intervals.[10] Calcium gluconate was available for intravenous injection, should extreme drowsiness appear.

9. Hawk, P. B.; Oser, B. L., and Summerson, W. H.: Practical Physiological Chemistry, ed. 12, Philadelphia, The Blakiston Company, 1947.

10. All the analyses for serum magnesium content were performed by Dr. Rees Pritchett.

Observation.—All 6 subjects experienced some degree of cramps of the lower part of the abdomen. In 3 of the subjects receiving one-half saturated solution, the rectal tube was out of the rectum some of the time, and it is certain that none of the 3 received the full 94 Gm. of magnesium sulfate. None of them exhibited symptoms or signs attributed to a central effect of magnesium. Subject A insisted on abandoning the experiment after 150 cc. of the enema had been given because of abdominal cramps. The serum magnesium level before the enema was 2.3 mg. per hundred cubic centimeters; at the time of discontinuation, 3.6 mg., and one hour later 2.8 mg. Subject B also had a control serum magnesium level of 2.3 mg. per hundred cubic centimeters, which rose to 3.9 mg. at two and one-half hours, at the end of the enema, and fell to 1.4 mg. one hour later. Subject D started with a level of 1.6 mg. per hundred cubic centimeters, rose to 3.4 mg. at two and one-half hours, the end of the enema, and fell to 3.0 mg. one hour later. Although in none of these 3 subjects did high levels of magnesium in the serum develop, and none exhibited central effects, it is to be noted that all showed some elevation of the magnesium level, with increases varying from 1.3 to 1.8 mg. per hundred cubic centimeters.

Subject C was successful in keeping the tube in the rectum throughout the three hours of his enema, and it may be assumed that he received the full 94 Gm. His initial serum magnesium level was 1.4 mg. per hundred cubic centimeters. After one and one-half hours the level was 3.3 mg. per hundred cubic centimeters, and the subject reported that he was beginning to feel warm all over. At two hours, he noted dryness of the lips and tongue. At two and one-half hours, the serum magnesium level was 7.0 mg. per hundred cubic centimeters, and the sensations of warm skin and dry mouth were intensified. Thirty minutes later, the enema was completed, and the subject remarked that the warm feeling was diminishing. One hour later, the magnesium level was 5.0 mg. per hundred cubic centimeters, and he claimed not to feel any different than usual. At no time did this subject display signs of drowsiness (chart 1).

Subject E received 250 cc. of a saturated solution of magnesium sulfate over a period of three hours, and since the rectal tube was retained it may be assumed that he received the full amount of 158 Gm. At the start, the serum magnesium was 1.5 mg. per hundred cubic centimeters, and the subject was conversing volubly, in his usual manner. At one hour and ten minutes, the serum level was 2.2 mg. per hundred cubic centimeters, and there were no symptoms. At one and one-half hours, the subject noted that his mouth was getting dry and his skin warm. At two hours and fifteen minutes, the serum magnesium was 7.0 mg. per hundred cubic centimeters, he said he was getting drowsy and there was a marked diminution in his speech activity. At three hours, the drip was stopped, after 158 Gm. of the salt had been admin-

istered, because the subject had become much more drowsy. The serum magnesium level was 12.1 mg. per hundred cubic centimeters. He lay quietly, breathing heavily and deeply, with eyes closed, and it required two or three commands or a rough shake to rouse him, on which he would speak thickly and slowly a few words and then fall asleep again. At this point, 1 Gm. of calcium gluconate was injected intravenously, and improvement was noted within one minute, it being easier to rouse him. Five minutes after the injection, he spontaneously remarked, "Now, I'm coming out of it." However, fifteen minutes later, he had

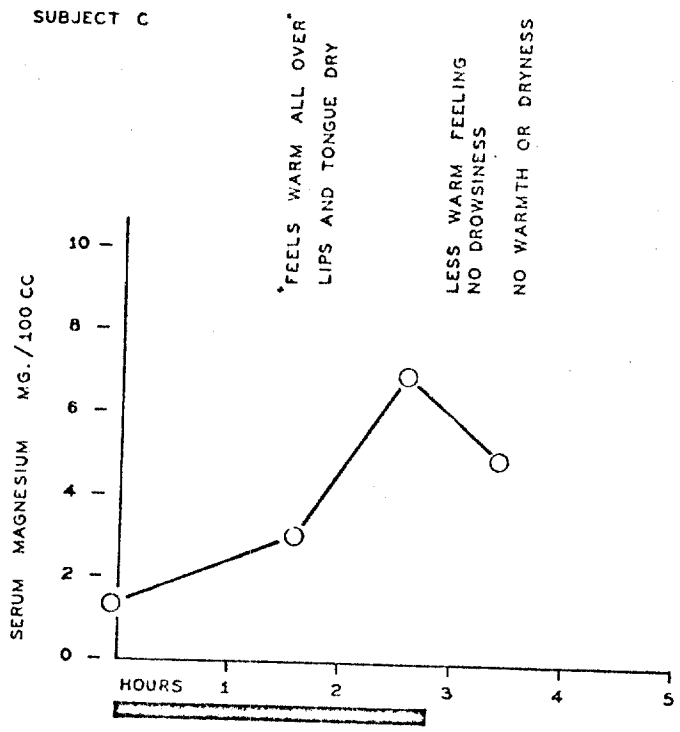


Chart 1.—Serum magnesium levels for subject C, who received per rectum 300 cc. of one-half saturated solution of magnesium sulfate, containing 94 Gm. of the salt.

lapsed into his previously depressed state, and another 1 Gm. of calcium gluconate was therefore administered. There was another slight improvement within five minutes; but it, too, was short lived, and he returned to a deep sleep, with snoring and deep respirations, from which he could be roused only with difficulty. Examination disclosed diaphragmatic respiration, active tendon reflexes and no pathologic reflexes. At this point the heart rhythm was grossly irregular, with a rate of about 100 a minute. The electrocardiogram showed auricular fibrillation. The subject slowly became less drowsy, and one hour after the

administration of the enema the serum magnesium level was 16.2 mg. per hundred cubic centimeters. A tap water enema was given, in order to evacuate any magnesium sulfate remaining in the rectum. Water was forced by mouth, and a high output of urine was maintained. The subject regained consciousness rapidly, passing in twenty minutes from a state of sleep, from which he could be roused to converse in thick speech, to a state of mild excitement, during which he sat up, talked in a loud manner, demanded to read the newspaper and called for food. He ate lunch, listened to the radio and remained in a state of relative excite-

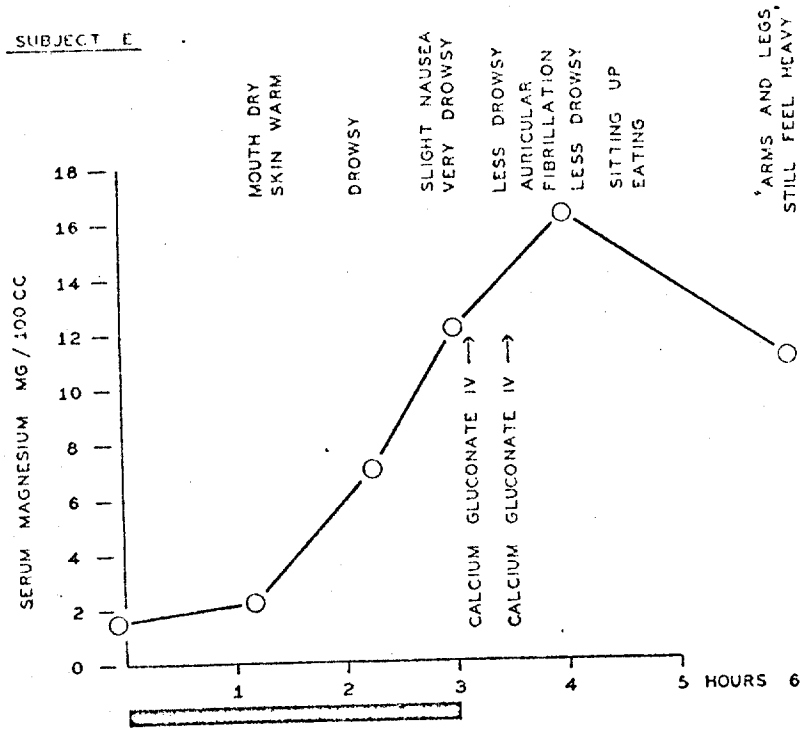


Chart 2.—Serum magnesium levels for subject E, who received per rectum 250 cc. of a saturated solution of magnesium sulfate, containing 158 Gm. of the salt.

ment for several hours. Three hours after the enema, the serum magnesium level had fallen to 10.9 mg. per hundred cubic centimeters, at which point the subject declared that his extremities felt heavy, but he was otherwise well (chart 2). However, auricular fibrillation was still present, and it persisted about four hours after it was first noted, when the rhythm reverted to normal.

Subject F received 200 cc. of saturated solution of magnesium sulfate over a period of two and one-half hours. It may be assumed that he received the whole 126 Gm. At the start, the serum magnesium level was 0.4 mg. per hundred cubic centimeters. Dry mouth and warm

skin were reported a full half hour before blood was drawn, which showed a serum magnesium level of 2.3 mg. per hundred cubic centimeters, and it is doubtful whether the early symptoms represented a true effect of magnesium in this patient, who was a medical student and anxious to perform well. However, after two hours and fifteen minutes, the serum level was 5.3 mg. per hundred cubic centimeters, and the mouth was reported drier and the skin very warm. He was slightly drowsy, stared listlessly at the window, allowed the newspaper to fall on the bed and occasionally closed his eyes. After two and one-half

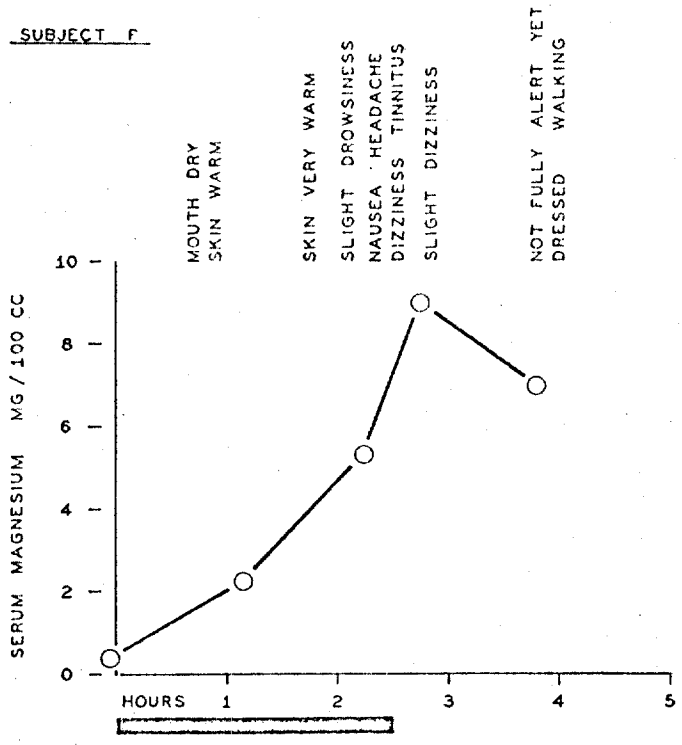


Chart 3.—Serum magnesium levels for subject F, who received per rectum 200 cc. of a saturated solution of magnesium sulfate, containing 126 Gm. of the salt.

hours, the drip was stopped, after 126 Gm. had been given, because of nausea, dizziness, a feeling of approaching syncope, headache and tinnitus. It is inferred that these symptoms were nonspecific, for they disappeared entirely in a few minutes. Ten minutes after the enema had been terminated, the serum magnesium was 9.0 mg. per hundred cubic centimeters, and the subject felt only slightly drowsy, with the same warm skin and dry mouth. All the symptoms gradually diminished, and one hour and twenty minutes after the end of the drip administration, the serum level was 7.0 mg. per hundred cubic centimeters.

and the subject felt well except for a feeling of apathy. He did not exhibit evidence of excitement (chart 3). It is of interest to note that neither of the 2 subjects who received the saturated solution of magnesium sulfate complained of local irritation.

SERIES 2.—Each of 3 subjects was given 40 cc. of saturated solution of magnesium sulfate by mouth, and specimens of blood were drawn at intervals thereafter. All of them showed some increase in the serum magnesium, the peak values being 4.0, 3.6 and 4.3 mg. respectively, per hundred cubic centimeters. It is suggested that some of the feeling of well-being so often experienced by persons who take a magnesium sulfate purge is secondary to a central sedative effect, rather than being due entirely to the relief of constipation.

COMMENT

From the evidence in the literature, and from the results reported in the preceding section, it is obvious that magnesium sulfate is absorbed from the gastrointestinal tract, including the rectum, in amounts sufficient to raise significantly the blood level and to produce depressant cerebral effects. Even normally functioning kidneys may not remove the salt from the blood stream fast enough to prevent the accumulation of amounts toxic to the central nervous system.

In our experience with 6 human subjects, the normal serum magnesium was in the range of 1.5 to 2.5 mg. per hundred cubic centimeters. Sensations of warm skin and dry mouth began at a level of about 4 mg. per hundred cubic centimeters. Drowsiness was noted at about 7 mg. per hundred cubic centimeters and became marked in the neighborhood of 12 mg. per hundred cubic centimeters. Between the levels of 12 and 16 mg., 1 subject was noted to exhibit diaphragmatic respirations, although they were maintained at average depth and rate. Respiratory arrest occurred only in the fatal case, in which the postmortem serum magnesium level was 20.1 mg. per hundred cubic centimeters. In 1 subject, a man aged 42 without evident heart disease, auricular fibrillation was present for about four hours during the highest levels of magnesium in the blood. It was not noted until after calcium had been administered, and the origin and significance of the abnormal rhythm are confused.

The total amount of magnesium sulfate U. S. P. administered may be roughly correlated with the height of the serum magnesium level reached. In 3 subjects given 25 Gm. of magnesium sulfate by mouth, the serum magnesium reached levels of approximately 4 mg. per hundred cubic centimeters, the highest being 4.3 mg. per hundred cubic centimeters. In the subject known to have received 94 Gm. of the drug per rectum, the serum magnesium reached a level of 7.0 mg. per hundred cubic centimeters; in the subject receiving 126 Gm. per rectum it

reached a level of 9.0 mg., and in the subject receiving 158 Gm. it reached a level of 16.2 mg. The patient receiving 189 Gm. had a postmortem level of 20.1 mg. per hundred cubic centimeters. The time factor was approximately constant, since all the enemas took about three hours. It is likely that the serum level reached with any given amount of magnesium sulfate will vary inversely with the duration of administration of the drug, the renal excretion and rectal leakage remaining constant.

It is inferred that an agent has been used in large amounts for oral and rectal administration with a sense of security that may not be justified. Magnesium sulfate may be absorbed rapidly, and dangerous concentrations in the blood may be reached even in the presence of normal renal function. For rectal administration, amounts of more than 94 Gm. over a three hour period are contraindicated. In amounts of this magnitude, or in smaller amounts given over a short period, as in a "magnesium, glycerin, water" enema, the patient should be carefully observed for signs of toxicity due to magnesium, namely, flushed skin with a feeling of warmth, dry mouth and, especially, increasing drowsiness. If the last sign appears, the procedure should be discontinued, the rectum washed out with tap water and fluids forced by mouth or vein. Calcium should be administered intravenously, although its effect as an antidote may be disappointingly temporary and repeated administration be necessary. The administration of magnesium sulfate by any route in a patient with impaired renal function is a hazardous procedure.

SUMMARY

A review of the literature on magnesium intoxication, together with a discussion of the pharmacology of magnesium sulfate, is presented.

The protocol of a patient exhibiting fatal magnesium intoxication is presented. The clinical phenomena of a second patient who, after receiving magnesium sulfate, became dangerously depressed are also described.

Hypertonic solution of magnesium sulfate was given per rectum to 6 male subjects, and significant absorption into the blood stream was demonstrated by measuring the serum levels. The latter were correlated with symptoms and signs of magnesium intoxication, notably a feeling of warm skin, dry mouth and drowsiness.

It has further been demonstrated that magnesium sulfate can be absorbed in large amounts from the intact bowel and may cause dangerous depression of the central nervous system, even in the presence of normal renal function. Limitations of the amount of magnesium sulfate to be administered are suggested, and a procedure is proposed for the management of a patient with signs of intoxication.

525 East Sixty-Eighth Street (Dr. Wolff).

Effect of Maternally Administered Magnesium Sulfate on the Neonate

STUART R. STONE, MD and JACK A. PRITCHARD, MD, FACOG

During a 14-year period, 7000 infants were born of mothers who had received magnesium sulfate parenterally because of preeclampsia or eclampsia. The serum level of magnesium of the fetus, although it rapidly approached that of the mother, could not be correlated with any ill effect. Magnesium sulfate was administered in intramuscular doses of 30–40 g during 24 hr to the gravida. This regimen was continued as long as she had demonstrable knee jerks, urine output of at least 100 ml during 4 hr, and no depression of respiration. No deleterious effect upon either fetus or newborn was noted.

SERIOUS COMPLICATIONS in newborn infants have been attributed by Lipsitz and English[1] to the toxic effects of maternally administered magnesium sulfate. They state that in the newborn of mothers so treated:

> One would anticipate dealing with a severely depressed infant at birth. The newborn may then require endotracheal resuscitation and often assisted ventilation for several hours. The cry would be noted to be absent or weak and the newborn would not respond to stimuli as normal infants do. The reflexes should be absent or feeble and the infant would require careful monitoring and intravenous fluids until the sucking reflex becomes stronger and the infant can receive oral feedings.

These authors did point out that the manifestations of acute or chronic intrauterine hypoxia could account for some of the findings, but they believed that their observations reflected the effects of excess magnesium on the already compromised newborn of a toxemic mother.

For the past 14 years magnesium sulfate has been the major therapeutic agent used in the management of toxemia of pregnancy at our hospital.[2] During that time more than 7000 infants were delivered whose mothers had received magnesium sulfate parenterally. A persistent clinical observation and one that contributed greatly to our enthusiasm for the use of magnesium sulfate in patients with severe toxemia of pregnancy was that magnesium sulfate effectively controlled the convulsions of eclampsia and prevented convulsions in preeclampsia without producing deleterious central nervous system depression of the mother, fetus, or newborn. Since the findings of Lipsitz and English appeared to be so contrary to our observations, we considered it imperative to evaluate critically the effects of maternal administration of magnesium sulfate on the newborn.

METHOD AND MATERIAL

Magnesium Sulfate Treatment Regimen

Initially the mother received 10 g of magnesium sulfate ($MgSO_4 \cdot 7H_2O$, USP) in a 50% solution injected intramuscularly, 5 g deeply into each buttock. Subsequently 5 g more in a 50% solution was administered intramuscularly in alternate buttocks every 4 hr as long as (1) the patellar reflex was present, (2) the urine output the preceding

From Obstetrics and Gynecology, The University of Texas (Southwestern) Medical School at Dallas and Parkland Memorial Hospital, Dallas, Tex.

Supported in part by Grant FR-05426, NIH.
Submitted for publication Sept 25, 1969.

4 hr was 100 ml or more, and (3) respirations were not depressed. For eclampsia, 4 g of magnesium sulfate in a 20% solution was injected intravenously over 3–4 min prior to the initial intramuscular injection of 10 g.[5]

Apresoline was administered intravenously in patients with severe hypertension. Barbiturates, large doses of narcotics, phenothiazine derivatives, and potent antihypertensive agents were avoided. Small doses of meperidine were used for analgesia during labor as indicated.

The impact on the newborn infant of maternally administered magnesium sulfate was evaluated as follows:

1. Blood was collected from the recently severed cords of 118 infants. Blood was also obtained from 42 of the mothers about the time of delivery. The serum was promptly separated and frozen until analyzed for its magnesium content. All measurements were performed in duplicate using an atomic absorption spectrophotometer. The Apgar score was determined 1 min after birth.

2. The neonatal mortality rate was determined for all patients with antepartum and intrapartum eclampsia cared for at our hospital during the past 14 years. All mothers had received magnesium sulfate prior to delivery.

3. The neonatal mortality rate was determined for all patients treated prior to delivery with magnesium sulfate during the past 2 years because of antepartum and intrapartum maternal hypertension. The number of mothers to receive magnesium sulfate prior to delivery was confirmed through detailed review of the hospital chart of every tenth patient delivered during that time. The maternal and newborn charts of every neonatal death during 1967 and 1968 were also carefully examined.

RESULTS

Magnesium Concentration and Apgar Score

The magnesium concentration of cord serum from the 118 infants ranged from 2.0 to 7.4 mEq per liter and averaged 3.7 mEq per liter. The magnesium concentration of the 42 maternal venous blood samples averaged 4.2 mEq per liter and ranged from 2.0 to 7.4 mEq per liter.

Only 8 of the 118 infants had 1-min Apgar scores of 5 or less. The 8 infants survived. Moreover, it is evident from Table 1

TABLE 1. CORD SERUM MAGNESIUM LEVELS OF NEWBORNS WITH LOW APGAR SCORES

Apgar score (1 min)	*Infant weight (g)*	*Cord Mg^{++} (mEq/liter)*
2	2180	4.6
3	1500	6.2
3	2470	3.9
3	3050	3.0
4	3610	3.1
5	2840	2.9
5	2650	3.6
5	3300	4.2

that there was not a positive correlation between cord serum magnesium concentration and Apgar score. The duration of therapy using the injection schedule outlined above did not influence materially either the cord or maternal serum magnesium concentration. The total dose of magnesium sulfate administered prior to delivery to the mothers of these 118 infants ranged from as little as 10 g to as much as 95 g. Figure 1 compares the mean cord and maternal serum magnesium concentrations to the total dose of magnesium sulfate administered before delivery. There was little difference in either cord or maternal levels regardless of whether the total dose prior to delivery was less than 20 g or more than 60 g.

Two of the 118 infants, or 1.7%, succumbed. One weighed 1375 g at birth and died on the third day of life. The 15-year-old mother had severe preeclampsia for which she had received 70 g of magnesium sulfate prior to delivery. The cord serum magnesium concentration was 3.8 mEq per liter. At autopsy minimal subarachnoid hemorrhage was identified. The other infant

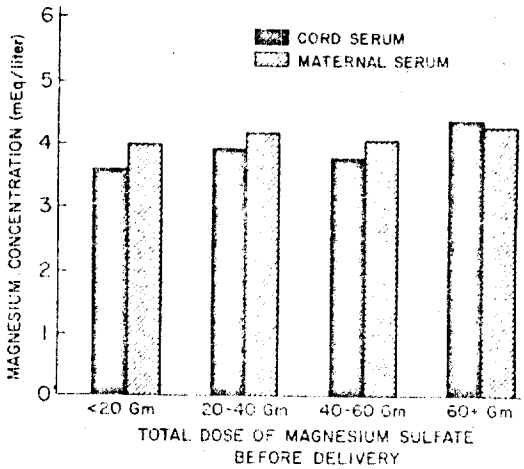


Fig 1. Mean serum magnesium concentrations in cord and maternal sera plotted according to the total dose of magnesium sulfate administered prior to delivery.

weighed 1950 g at birth and succumbed following futile attempts to repair a large meningomyocele. Seventeen low-birth-weight infants—ie, less than 2500 g—all survived, as did the 99 infants classified as mature by virtue of a birth weight of at least 2501 g.

Neonatal Mortality in Eclampsia Treated with Magnesium Sulfate

Since 1955, 80 infants have been delivered from mothers with eclampsia diagnosed and treated with magnesium sulfate prior to delivery. Every fetus in whom a fetal heart beat was identified at the time that eclampsia was diagnosed and treatment with magnesium sulfate was instituted was delivered alive. In turn, every liveborn infant that weighed 1800 g (4 lb) or more survived. The smallest infant to survive weighed 896 g. The infant's mother had received 49 g of magnesium sulfate prior to delivery. The fate of each of the 80 fetuses is summarized in Table 2.

Neonatal Mortality During 1967–1968

Almost exactly 10% of 12,024 mothers who were delivered in 1967 and 1968 at our hospital received magnesium sulfate parenterally prior to delivery because of hypertension complicating the pregnancy. Twenty-four of the 1248 infants of these hypertensive mothers succumbed during the newborn period, a neonatal death rate of 1.9%. Twenty of the 24 infants that succumbed weighed less than 1800 g (4 lb). Pertinent data regarding the 4 deaths of infants weighing 1800 g or more are presented in Table 3.

TABLE 2. FATE OF 80 FETUSES OF WOMEN WITH ANTEPARTUM ECLAMPSIA TREATED WITH MAGNESIUM SULFATE

	Infant survival			
	All infants		*Alive at time of diagnosis*	
Weight (g)	*(No.)*	*(%)*	*(No.)*	*(%)*
<1000	1/7	15	1/5	20
1000–1500	3/6	50	3/5	60
1500–2000	8/11	73	8/11	80*
2000 or more	53/56	95	53/53	100
TOTAL	65/80	81	65/73	89

* All infants weighing 1800 g (4 lb) or more survived.

TABLE 3. NEONATAL FATALITIES DURING 1967–1968 FOR INFANTS WEIGHING 1800 G OR MORE

Infant	*Weight*	*Comment*
E.J.	1815	Extensive placental abruption
M.M.	1950	Large meningomyelocele; repair unsuccessful
G.G.	2240	Ruptured omphalocele and perforated bowel
M.W.	2440	Precipitous labor and delivery; marked meconium staining; aspiration pneumonitis

The neonatal death rate for all infants delivered in 1967 and 1968 was 301 out of 11,897 live births or 2.5%. Thus the neonatal death rate for the total newborn population was nearly one-third greater than for infants whose mothers had received magnesium sulfate because of hypertension in pregnancy.

DISCUSSION

Parenterally administered magnesium sulfate has been used extensively in the United States as an integral part of treatment of

toxemia of pregnancy. Several large teaching centers employ this drug as the primary therapeutic agent.[6] Therefore the published warning of Lipsitz and English made mandatory further careful evaluation of the safety of this drug in spite of previous reports pointing out the lack of deleterious effects on both the mother and her infant.[4,5]

The studies reported now establish that magnesium sulfate does not effect deleteriously the newborn, at least when administered primarily by intermittent deep intramuscular injection in doses of 30–40 g per 24 hr and only as long as maternal knee jerks are demonstrable, urine flow is at least 100 ml for 4 hr, and respirations are not depressed. Actually the neonatal mortality rate was somewhat less for infants whose mothers were so treated than for the general neonatal population at our hospital.

The observations of Lipsitz and English that parenteral administration of magnesium sulfate to the mother prior to delivery raises the serum magnesium level in the fetus and newborn confirms what seems to have been well established.[1-4] Previous studies, as well as those reported now, also indicate the serum level in the fetus and the newborn soon approaches the level of the mother. This in itself is hardly proof that the magnesium ion administered to the mother in therapeutically effective doses is deleterious to the newborn. Moreover, the failure to establish any consistent correlation between the magnesium level in the newborn and the Apgar rating or with neonatal mortality in the report of Lipsitz and English as well as ours implies at least that factors other than the magnesium ion contributed significantly to the compromised states described by them in several newborn infants.

If signs of impending maternal magnesium toxicity were to be disregarded no doubt magnesium sulfate could be administered to the mother in doses harmful to the fetus and newborn infant. Our observations indicate, however, that magnesium sulfate administered according to the dosage schedule and safeguards reiterated in this report effectively prevents convulsions in women with preeclampsia. In mothers with eclampsia it nearly always stops convulsions, prevents further convulsions, and allows the mothers to regain consciousness soon without deleterious central nervous system depression.[5] The observations reported now concerned with the fate of the newborn infants of women treated in this way confirms our longstanding impression that magnesium sulfate so administered is not harmful and may be beneficial to the newborn infant. Moreover, the benefits to the fetus and newborn need not be limited just to those derived from the prevention of convulsions in the mother. Harbert *et al*, for example, have reported increased uterine blood flow in pregnant hypertensive subhuman primates in response to magnesium sulfate administration.[2]

CONCLUSION

No evidence was obtained that magnesium sulfate administered prior to delivery to hypertensive mothers according to a standardized dosage schedule that effectively prevented eclamptic convulsions was deleterious to their infants.

The University of Texas Southwestern Medical School at Dallas
5323 Harry Hines Blvd
Dallas, Tex 75235

REFERENCES

1. Chesley LC, Tepper I: Plasma levels of magnesium attained in magnesium sulfate therapy for preeclampsia and eclampsia. *Surg Clin N Amer 37*:353, 1957
2. Harbert GM Jr, Cornell GW, Thornton WN Jr: Effect of toxemia therapy on uterine dynamics. *Amer J Obstet Gynec 105*:94, 1969
3. Lipsitz PJ, English IC: Hypermagnesemia in the newborn infant. *Pediatrics 40*:856, 1967
4. Pritchard JA: The use of the magnesium ion in the management of eclamptogenic toxemias. *Surg Gynec Obstet 100*:131, 1955
5. Pritchard JA, Stone SR: Clinical and laboratory observations on eclampsia. *Amer J Obstet Gynec 99*:754, 1967
6. Zuspan FP: Treatment of severe preeclampsia and eclampsia. *Clin Obstet Gynec 9*:954, 1966

J. Am. Med. Assoc. 88(13): 1186-1188 (1927)

Clinical Notes, Suggestions and New Instruments

A CASE OF MAGNESIUM SULPHATE POISONING*

HARVEY S. THATCHER, M.D., LITTLE ROCK, ARK.

Death from magnesium sulphate poisoning is rarely reported. It is a recognized fact that magnesium sulphate in large doses is toxic, but few cases of death from this drug with autopsy reports have been recorded.

L. M. S., an American farmer, aged 26, poorly nourished, single, entered the State Hospital for Nervous Diseases, Jan. 11, 1927, with a provisional diagnosis of psychoneurosis of the neurasthenic type. At 6 a. m., January 12, he was given a dose of magnesium sulphate. A solution of the salt had been placed accidentally in the same ward near the patient. The preparation had disappeared and evidently had been taken by him. He was found dead at 7:10 a. m.

The principal gross pathologic changes were the presence of approximately 1 liter of yellowish brown fluid in the stomach, and a dark red and hemorrhagic appearance of the lining of the stomach. There were recent hemorrhages throughout the small intestine, and considerable blood was mixed with the contents. The hemorrhages averaged 5 mm. in their largest dimension. There were a few hemorrhages in the region of the cecum. There was marked congestion of the lungs, trachea and main bronchi, and of the heart, liver and kidneys. There was also acute splenic hyperplasia.

The principal microscopic pathologic changes were small hemorrhages on the mucosa of the stomach and the intestine. The lungs were markedly congested. The vessels of the heart, kidneys, spleen, liver, stomach, intestine and suprarenals were engorged with blood.

The chemist reported 883½ grains (57 Gm.) of magnesium sulphate in the contents of the stomach. As the dosage is 240 grains (15.5 Gm.), the amount recovered was more than three times the required dose.

Boos[1] collected reports of ten cases of magnesium poisoning in 1911. In only three of these had autopsy been performed. The main postmortem and operative observations were "patches of reddening on the mucosa of the stomach with free fluid in the abdominal and pleural cavities." Since that date Prestley[2] and Anderson[3] have reported two cases each of similar poisoning with recovery. There must have been an idiosyncrasy to the drug in the first patient reported by Prestley. The dosage is not given for his second patient. The dosage was entirely too large for Anderson's patients. Kerr[4] reported two cases of tetanus in children resulting from subcutaneous injections of magnesium sulphate because of the depression of the muscular and nervous functions. Respiratory paralysis and death occurred. The dosage must have been too large.

The case reported here illustrates that more care should be exercised in the administration of magnesium sulphate. Toxicity may result without death. An idiosyncrasy may exist and the average dose may be toxic.

If toxicity does occur after its use, the treatment consists of the subcutaneous or intravenous administration of calcium salts, according to Meltzer and Auer.[5] There is an antagonistic action of calcium on the inhibitory effect of magnesium.

---

* From the Department of Pathology, University of Arkansas School of Medicine.

1. Boos, W. F.: Magnesium Poisoning: A Study of Ten Cases, J. A. M. A. 55: 2037-2042 (Dec. 10) 1910.
2. Prestley, J. P.: Toxic Effects of Magnesium Sulphate, New York M. J. 96: 665 (Sept. 28) 1912.
3. Anderson, W. W.: Magnesium Sulphate Poisoning in Children, J. M. A. Georgia 10: 826-830 (Dec.) 1921.
4. Kerr, LeGrand: Case Reports: Two Experiences with the Subcutaneous Injection of Sulphate of Magnesium in Tetanus in Children, Long Island M. J. 8: 90-92 (March) 1914.

MEDICAL PROGRESS

MAGNESIUM METABOLISM*

WARREN E. C. WACKER, M.D., AND ALFRED F. PARISI, M.D.

OVER the decade since this subject was last reviewed in the *journal*[1] much has been added to the knowledge of normal human magnesium metabolism and of its alteration in disease. This has ensued largely because of the advances made in the quantitative analysis of this element in biologic samples. A major step forward has been the successful use of flame methods to measure magnesium in body fluids and tissues. Application of the techniques of emission spectroscopy led to the perfection of a multichannel flame spectrometer that gave precise and accurate determinations of the relatively small amounts of magnesium in serum, urine and tissues samples.[2,3] The more recent development of atomic absorption spectroscopy provides instrumentation for general use whereby multiple determinations of minute amounts of magnesium can be reliably made on small samples in a matter of minutes.[4,5]

The availability of this simple, precise method has already led to a better understanding of the role of magnesium metabolism in human disease, a process that should accelerate in the next few years.

BODY CONTENT, ABSORPTION AND EXCRETION

The adult human body contains 24 to 28 gm, or approximately 2000 mEq, of magnesium.[11] It is the fourth most abundant cation in the body, and the second most plentiful intracellularly. Bone contains about half the total body magnesium, the remainder being almost equally distributed between muscle and the nonmuscular soft tissues.[12] Of the nonosseous tissues, liver and striated muscle have the highest concentrations, 15-20 mEq per kilogram.[13] The magnesium content of erythrocytes varies from 4.4 to 6.0 mEq per liter.[14,15]

Owing to the multiplicity of imprecise methods for the determination of magnesium, normal serum values have ranged from as low as 1.1 and as high as 2.5 mEq per liter.[3,12] Atomic absorption methods show that mean values of normal serum vary between 1.6 and 2.1 mEq per liter.[9,16-18] Careful assessment of the normal serum content by two different analytical methods and certified standards has yielded a value close to 2.0 mEq per liter.[9] Differences in the control populations may account for some variation. It is important to point out that in all cases, limits of the normal range deviate from the mean by less than 15 per cent, indicating that the serum concentration is maintained by sensitive control mechanisms that are poorly understood at present.

The average daily intake of a human adult on a normal diet is of the order of 25 mEq, much of which is obtained from green vegetables, which have large amounts as part of chlorophyll. Recent studies have shown that an intake of 0.30 to 0.3 mEq per kilogram of body weight per day is required to maintain positive magnesium balance in normal persons.[19]

Stool content varies with intake and with the dietary content of anions, particularly phosphate. Calcium also influences the amount of magnesium absorbed, apparently by competition for a common absorptive pathway.[20]

Animal studies indicate that magnesium is absorbed mainly in the small intestine.[21] In man the time course of appearance of orally administered labeled magnesium ^{28}Mg in plasma is consistent with this interpretation. Peak levels of the isotope are measured between two and eight hours after the dose.[22] However, colonic absorption can occur, as evidenced by hypermagnesemia developing after rectal enemas.[23,24]

Normally about a third of the ingested magnesium is absorbed and excreted in the urine. Although this figure provides a good estimation under usual dietary circumstances, the total dietary intake of the element must be considered. In a study of three subjects given oral ^{28}Mg, 44.3 per cent of diet containing 20 mEq per day was absorbed, whereas 75 per cent of a diet containing 1.9 mEq and 53.7 per cent of one containing 47 mEq per day were absorbed.[22]

When ^{28}Mg is given intravenously to normal subjects, 1 to 2 per cent is recovered in the feces.[25] These observations support the contention that little endogenous magnesium is eliminated by the fecal route under ordinary circumstances.

Under conditions of magnesium deprivation renal conservation becomes prominent, restricting urinary magnesium losses to less than 1 mEq per day.[27] There is usually a concomitant diminution in fecal excretion. However, in the most recent study of depletion in human beings fecal excretion exceeded intake despite significant renal conservation. This was attributed to the 30 to 70 mM [illegible]

*From the Biophysics Research Laboratory, Department of Biological Chemistry, Harvard Medical School, and the Division of Medical Biology, Department of Medicine, Peter Bent Brigham Hospital, Boston. Address reprint requests to Dr. Wacker at the Department of Medicine, Peter Bent Brigham Hospital, Boston, Mass. 02115.

Original work contained in this review was supported by a venture grant from The Nutrition Foundation, Incorporated, and by a grant-in-aid (HE-07297) from the National Institutes of Health, Public Health Service, [illegible] Department of Health, Education, and Welfare.

contained in the experimental diet, which presumably interfered with the reabsorption of endogenous magnesium contained in the intestinal secretions.[31]

Biochemistry of Magnesium

Magnesium is an activator in vitro of a host of enzyme systems that are critical to cellular metabolism. Prominent are the enzymes that hydrolyze and transfer phosphate groups, among them the phosphatases and those concerned in the reactions involving adenosine triphosphate. Since adenosine triphosphate (ATP) is required for glucose utilization, fat, protein, nucleic acid and coenzyme synthesis, muscle contraction, methyl group transfer, sulfate, acetate and formate activation, by inference the activating effect of magnesium extends to all these functions.

Additionally, magnesium is required as a cofactor for oxidative phosphorylation in vitro.[32,33] Attempts to demonstrate an in vivo counterpart of this requirement on mitochondria obtained from magnesium-deficient animals have led to conflicting results.[34]

Magnesium contributes importantly to macromolecular structure. The highly ordered organization of DNA,[35,36] RNA and ribosomes[36,37] is stabilized by the presence of this metal. Maximum stabilization of DNA to thermal disruption occurs when a 1:1 stoichiometry is reached between equivalents of magnesium ion and DNA phosphate residues.[35]

Magnesium is necessary for the structural integrity of ribosomal particles from a variety of sources. The aggregation or dissociation of component particles in ribosomes obtained from *Escherichia coli*,[38,41] baker's yeast,[42] pea seedlings,[43,44] rat liver[45] and rabbit reticulocytes[46] is critically dependent on the ambient magnesium concentration. Thus, crude extracts of exponential cultures of *E. coli* made in 0.01 M Mg^{++} predominantly contain a 100S ribonucleoprotein. In 0.001 M Mg^{++} the major ribonucleoprotein component has a sedimentation constant of 70S. With 0.0001 M Mg^{++} ribonucleoprotein particles were present as 30S and 50S peaks only. Additional study showed that each 70S component is made of a 30S and 50S subunit, and that the 100S particle is a dimer of two 70S subunits.[38,41] The fully separated subunits will reaggregate when the magnesium concentration is increased. The analogous role of magnesium in plant and animal systems cited and the generality of this phenomenon suggest its fundamental importance in cellular function.

Magnesium is further involved in protein synthesis by contributing to the binding of messenger RNA to the 70S ribosome.[47,48] Similar findings were obtained for the interaction of sRNA with a site on the 50S subunit of the 70S ribosome. At 0.01 M Mg^{++} the sRNA is bound to ribosomes whereas at 0.0001 M Mg^{++} no association is observed.[49]

Magnesium is also required for the in vitro synthesis[50] and degradation of DNA.[51] The metal has also been included in all the amino acid activating systems.[52,53] The formation of aminoacyl sRNA involves two distinct steps:

I. *Activation*

$$\text{Enzyme} + \text{Amino Acid} + \text{ATP} \overset{Mg^{++}}{\rightleftharpoons} \text{Enzyme-Amino-Acyl-Adenylate} + PP_i$$

II. *Transfer*:

$$\text{Enzyme-Amino-Acyl-Adenylate} + \text{sRNA} \rightleftharpoons \text{Amino Acyl sRNA} + \text{Enzyme} + \text{AMP}.$$

The first step is the amino acid activation step in which a *specific* amino acyl sRNA synthetase forms a complex with its amino acid in the presence of ATP. This step is known to be magnesium dependent.[54,55] Optimal activity of each of the amino acyl RNA synthetases occurs at well defined Mg:ATP ratios.[57] In the second step or transfer reaction a specific sRNA accepts its amino acid from the enzyme complex. This step was originally reported not to have a magnesium requirement.[56,58] However, if the sRNA has been prepared in a manner likely to remove metals that it naturally contains — for example, dialysis against chelating agents — readdition of magnesium or other divalent cations may become necessary for the transfer reaction. Thus, threonyl sRNA from *E. coli*, yeast and rat liver[59] and leucyl sRNA from yeast[60] denatured in this manner show maximal biologic activity for accepting their respective amino acids upon reconstitution with magnesium.

The observations point out the more general problem of evaluation of the chemical role of a metal in a biologic system. Direct analysis of endogenous magnesium as well as other divalent metals in such systems is rarely undertaken. Thus, whereas the role of magnesium appears critical, the information necessary to evaluate its specificity in a decisive way is usually not available. Nevertheless, as the most abundant divalent intracellular cation, analogous in vivo function is implied as the most tenable hypothesis.

In this regard it is important to point out some recent observations on the interrelations of the major biologic cations.[63] It is well known that potassium is generally found to be present in high concentration within cells whereas the intracellular sodium content is low. These ratios are inverted in extracellular fluids. A similar relation between the intracellular and extracellular concentrations of magnesium and calcium exists. As shown by Figure 1, the intracellular ratios log $[K^+]/[Na^+]$ and log $[Mg^{++}]/[Ca^{++}]$ are related. In erythrocytes there is a direct relation between K^+/Na^+ and Mg^{++}/Ca^{++} and an inverse relation between K^+/Mg^{++} and Na^+/Ca^{++}. Similar relations hold in other mammalian cells (Fig. 2). In general, the higher the metabolic activity of a cell, the greater the ratios $[Mg^{++}]/[Ca^{++}]$ and

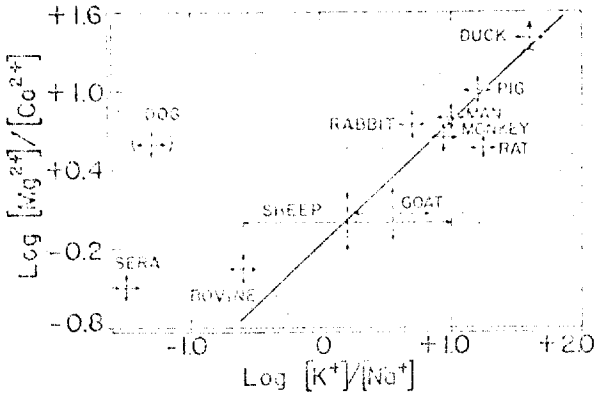


FIGURE 1. *Relation between the Ratio of Monovalent and Divalent Ion Concentrations in Erythrocytes.*

The ratio in dog erythrocytes is anomalous; while sodium and calcium are both high, magnesium is also high.

$[K^+]/[Na^+]$. This is apparent in Figure 1 for the erythrocytes of ducks, which are nucleated and therefore have greater metabolic activity than the anuclear mammalian red cells, and in Figure 2, where the ratios are higher in brain and skeletal muscle than in relatively inactive tissues such as skin.

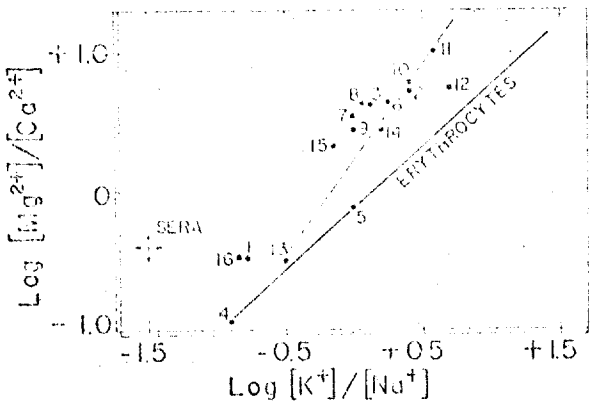


FIGURE 2. *Relation between the Concentration Ratio of Monovalent Ions and Divalent Ions in Different Organs and Cells.*

1 indicates smooth muscle (pig), 2 smooth muscle (frog), 3 uterine muscle (cat), 4 bladder muscle (pig), 5 uterine muscle (man), 6 cardiac muscle (man), 7 skeletal muscle (man, vastus), 8 skeletal muscle (pig, sartorius), 9 skeletal muscle (rabbit, soleus), 10 skeletal muscle (man, adult), 11 skeletal muscle (pig, adult), 12 skeletal muscle (rabbit, adult), 13 skin (man), 14 brain (man), 15 spinal cord (man), and 16 amniotic fluid (man).

Another striking relation obtains between the intracellular concentration of magnesium and the content of phosphorus (Fig. 3). As the intracellular content of magnesium and calcium increases (magnesium representing the major part of this sum) from inactive tissue, such as skin and erythrocytes, up to very active tissue, such as liver, brain and striated muscle, there is a concomitant increase in the cellular content of phosphorus. Conversely, the sum of $[K^+ + Na^+]$ does not increase. These striking relations are in keeping with the known in vivo

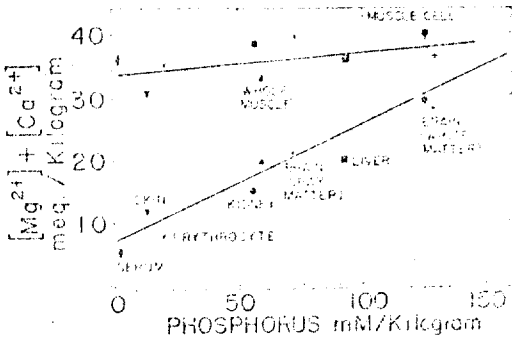


FIGURE 3. *Relation between Total Monovalent Ion (Upper Line) Total Divalent Ion (Lower Line) and Total Phosphorus in Different Human Organs and Cells.*

requirements of magnesium both as an activator of most of the enzymes involved in phosphorus metabolism and as an agent in mitochondrial oxidative phosphorylation. The exquisite control of these ion concentrations and the energy expended in maintaining the ion gradients strongly suggest the biologic importance of magnesium in these systems.

PHYSIOLOGIC AND PHARMACOLOGIC CONSIDERATIONS

Magnesium and calcium have complex interdependent influences on the excitability of the components of the neuromuscular apparatus. Magnesium or calcium depletion leads to increased neuronal excitability and enhanced neuromuscular transmission. In muscle, however, the effects of calcium and magnesium are antagonistic. In the intact animal the overall effect of either low calcium or low magnesium is increased neuromuscular excitability.[59]

In pharmacologic doses magnesium has a curariform action on the neuromuscular junction,[61] presumably by interfering with the release of acetylcholine from motor-nerve terminals.[62]

Direct application of magnesium to central nervous tissue blocks central synaptic transmission and may cause general anesthesia.[63,64] It is generally accepted that parenteral administration of magnesium salts to human beings can cause narcosis if given in sufficient quantities.[64] The chief experimental evidence supporting this contention is the report of Peck and Meltzer[65] on the ability to anesthetize and perform surgery on three patients with the use of intravenous magnesium as the sole narcotizing agent. Somjen et al.[66] have recently challenged these observations. Two persons were given intravenous magnesium sulfate sufficient to raise the serum level to 15 mEq per liter and cause neuromuscular paralysis; however, they did not lose consciousness or pain perception. A definitive resolution of these apparently conflicting observations is not evident. Since some depression of respiratory function was reported in both studies the possible role of anoxia or hypercapnia under these circumstances becomes an important consideration.

Magnesium Deficiency in Animals

Experimental magnesium deficiency has been produced in many animal species. Many of the data pertain to work on rats, but magnesium deficiency has frequently been observed in sheep and cattle, species in which the syndrome develops spontaneously.

In 1938 Ernse, Orent and McCollum[30] induced magnesium deficiency in weanling rats by feeding a diet containing 0.09 mEq per liter of the element. In these animals hyperemia and progressive neuromuscular irritability developed, culminating in generalized and sometimes fatal seizures.

These early experiments have been confirmed and extended by many workers. Hypomagnesemia, accompanied by diminution in red cell, muscle, bone and total carcass magnesium[3,16,17,31-33] occurs.

Magnesium deficiency has a profound influence on other metals and metabolites. In adult rats hyperkalemia, decrease in potassium and increase in sodium in muscle and liver and azotemia have been reported.[17,32,33] Soft tissue potassium depletion and sodium increase have been correlated with loss of cellular nonfollicular nitrogen, indicating cellular damage.[33] Available evidence suggests that with sufficient magnesium depletion the pumping mechanisms that maintain normal intracellular Na^+ and K^+ are inactivated, resulting in these changes.

The mechanism of hypercalcemia in these circumstances is elusive. Although it is tempting to invoke increased parathyroid function, there is no concomitant hypophosphatemia[1] or decrease in bone calcium.[7] The azotemia is also unexplained. Glomerular filtration rates were not determined in these studies. Among the multiple pathologic lesions nephrocalcinosis, myocardial necrosis and calcification and lipid deposition in the aorta have been most striking.[3,16,32,33,34,35]

Hypomagnesemic tetany occurs spontaneously in calves fed for a long time on a pure milk diet.[3,16,17,36] A gradual reduction of serum magnesium results in hyperirritability and tetany, the latter appearing at concentrations below 1 mEq per liter. Defective absorption of magnesium has been documented as this diet is maintained.[3,37] Magnesium supplements prevent the disease.[3,38]

It should be noted that milk is a relatively poor dietary source of magnesium. Fresh whole cow's milk contains 11.5 mEq per kilogram, whereas human milk contains 1.9 mEq per kilogram. Most meats, grains and raw green vegetables have magnesium in excess of 20 mEq per kilogram.[3,39]

A second and more puzzling form of magnesium deficiency known as "grass tetany" occurs in adult lactating sheep and cattle.[40] Neuromuscular irritability, tetany and convulsions develop in the affected animals, sometimes within two days, when they are turned out to graze in lush pastures in the spring. Although hypomagnesemia usually develops, there is no evidence of depletion of tissue stores of the element. Again, magnesium administration can reverse or prevent the disease.[3,3,35] The acute hypomagnesemia is thought to be related to peculiar conditions of their forage, and may be due to fertilization with ammonium salts. Severe hypomagnesemia will develop in a few days in lactating cows given artificial rations low in magnesium unless magnesium supplements are provided.

The regulation of serum magnesium under these circumstances appears critically dependent on the daily intake, a phenomenon not found in other species. Magnesium content of grass from heavily fertilized fields does not differ from that of normal pastures. The protein content of grass from fields topdressed with ammonium salts is increased, however, and this has been implicated as a factor causing grass tetany.[36]

In view of the absence of magnesium depletion it appears that there is a failure to mobilize tissue stores under these circumstances. In animals the serum magnesium concentration at which tetany appears varies with the nutritional status. Thus, when sheep are made magnesium deficient, then treated by magnesium administration and allowed to become deficient a second time, tetany occurs only after the serum magnesium has fallen considerably below the level at which it occurred the first time.[37] This observation has important implications for human magnesium deficiency since the onset of symptoms cannot be correlated directly with the concentration of magnesium in serum.

(To be continued)

References

1. Wacker, W. E. C., and Vallee, B. L. Magnesium metabolism. *New Eng. J. Med.* **259**:431-438 and 475-482, 1958.
1a. *Idem.* Magnesium. In *Mineral Metabolism: An advanced treatise. Vol. 2. The elements, Parts A-B.* Part A. Edited by C. L. Comar and F. Bronner. New York: Academic Press, 1964. Pp. 483-521.
2. Vallee, B. L. Simultaneous determination of sodium, potassium, calcium, magnesium, and strontium by new multichannel flame spectrometer. *Nature* (London) **173**:1050, 1954.
3. Wacker, W. E. C., and Vallee, B. L. Study of magnesium metabolism in acute renal failure employing multichannel flame spectrometer. *New Eng. J. Med.* **257**:1258-1262, 1957.
4. Walsh, A. Application of atomic absorption spectra to chemical analysis. *Spectrochim. acta* **7**:108-117, 1955.
5. Willis, J. B. Determination of metals in blood serum by atomic absorption spectroscopy. II. Magnesium. *Spectrochim. acta* **16**:273-278, 1960.
6. Dawson, J. B., and Heaton, F. W. Determination of magnesium in biological materials by atomic absorption spectrophotometry. *Biochem. J.* **80**:99-106, 1961.
7. Horn, D. B., and Lamer, A. L. Estimation of magnesium by atomic absorption spectrophotometry. *Clin. chim. acta* **8**:974-976, 1963.
8. Stewart, W. K., Hutchinson, F., and Fleming, L. W. Estimation of magnesium in serum and urine by atomic absorption spectrophotometry. *J. Lab. & Clin. Med.* **61**:858-872, 1963.
9. Wacker, W. E. C., Iida, C., and Fuwa, K. Accuracy of determinations of serum magnesium by flame emission and atomic absorption spectrometry. *Nature* (London) **202**:659-661, 1964.
10. Iida, C., Fuwa, K., and Wacker, W. E. C. General method for magnesium analysis in biological material by atomic absorption spectroscopy. *Analytical Biochem.* **18**:18-26, 1967.
11. Widdowson, E. M., McCance, R. A., and Spray, C. M. Chemical composition of human body. *Clin. Sci.* **10**:123-133, 1951.
12. Aikawa, J. K. *The Role of Magnesium in Biologic Processes.* Springfield, Illinois: Thomas, 1963.

13. Arkawa, J. K.: Effect of glucose and insulin on magnesium metabolism in rabbits: study with Mg^{28}. *Proc. Soc. Exper. Biol. & Med.* **103**:363-366, 1960.
14. Vallee, B. L., Holt, J. M., Parlson, R., and Sziveck, J. Spectrochemical analysis of sodium, potassium, calcium, magnesium, copper and zinc in normal human erythrocytes. *J. Clin. Investigation* **44**:689-695, 1965.
15. Seller, R. H., Fenster, O., Bell, A. N., and Meyer, J. H. Serum and erythrocyte magnesium levels in congestive heart failure: effect of hyperventilation-alkalosis. *Am. J. Cardiol.* **17**:786-791, 1966.
16. Alcock, N., McIntyre, I., and Radde, I. Determination of magnesium in body fluids and tissues by flame spectrophotometry. *J. Clin. Path.* **13**:509-510, 1960.
17. Wacker, W. E. C., Ulmer, D., and Fuwa, K. Concentration of magnesium in normal plasma or serum. *Nature* (London) **205**:690, 1965.
18. Brisco, A. M., and Kenyon, C.: Relation of magnesium to calcium in human blood serum. *Nature* (London) **214**:1126, 1967.
19. Jones, I. T., Mendlo, R., and Clark, I. B. Magnesium requirements in adolescence. *J. Clin. Nutrition* **20**:632-635, 1967.
20. Alcock, N., and MacIntyre, I. Interrelation of calcium and magnesium absorption. *Clin. Sci.* **22**:185-193, 1962.
21. Arkawa, J. K. Gastrointestinal absorption of Mg^{28} in rabbits. *Proc. Soc. Exper. Biol. & Med.* **100**:293-295, 1959.
22. Graham, L. A., Caesar, J. J., and Burgen, A. S. V. Gastrointestinal absorption and excretion of Mg^{28} in man. *Metabolism* **9**:659-659, 1960.
23. Stevens, A. R., Jr., and Wolf, H. G. Magnesium intoxication: absorption from intact gastrointestinal tract. *Arch. Neurol.* **63**:749-759, 1950.
24. Fawcett, D. W., and Gross, J. P. Magnesium poisoning following enema of epsom salt solution. *J. A.M.A.* **123**:1025, 1943.
25. Silver, I., Rosenbaum, J. S., and Dahl, L. K. Magnesium turnover in human osteoporosis. *J. Clin. Investigation* **39**:420-425, 1960.
26. Arkawa, J. K., Gordon, G. S., and Rhodes, F. L. Magnesium metabolism in human beings: studies with Mg^{28}. *J. Appl. Physiol.* **15**:557-559, 1960.
27. Fitzgerald, M. G., and Fourman, P. Experimental study of magnesium deficiency in man. *Clin. Sci.* **15**:635-647, 1956.
28. Barnes, B. A., Cope, O., and Harrison, L. Magnesium conservation in normal man on low magnesium diet. *J. Clin. Investigation* **37**:433-438, 1958.
29. Barnes, B. A., Cope, O., and Gordon, E. B. Magnesium requirements and depletion: evaluation of two surgical patients. *Ann. Surg.* **152**:418-433, 1960.
30. Shils, M. E. Experimental human magnesium depletion. I. Clinical observations on blood chemistry alterations. *Am. J. Clin. Nutrition* **15**:133-143, 1964.
31. Duee, M. J., and Walser, M. Magnesium depletion in normal man. *Metabolism* **15**:684-695, 1966.
32. Potter, V. R. Assay of animal tissues for respiratory enzymes. IV. Cytochrome c in relation to fatty acid oxidation. *J. Biol. Chem.* **163**:337-346, 1946.
33. Elvehjem, A. Assay of animal tissues for respiratory enzymes. VI. Further studies on oxidative phosphorylation. *J. Biol. Chem.* **160**:17-37, 1947.
34. Lehninger, A. L., and Kennedy, E. P. Requirements of fatty acid oxidase complex of rat liver. *J. Biol. Chem.* **173**:753-771, 1948.
35. Vitale, J. J., Nakamura, M., and Hegsted, D. M. Effect of magnesium deficiency on oxidative phosphorylation. *J. Biol. Chem.* **223**:573-576, 1957.
36. Beeckley, R. B., Clewes, N. W., and MacIntyre, I. Oxidative phosphorylation in magnesium and potassium deficiency in rat. *Am. J. Physiol.* **201**:1170-1172, 1961.
37. Dove, W. F., and Davidson, N. Cation effects on denaturation on DNA. *J. Molec. Biol.* **5**:467-478, 1962.
38. Venner, H., and Zimmer, C. Studies on nucleic acids. VIII. Changes in stability of DNA secondary structure by interaction with divalent metal ions. *Biopolymers* **4**:321-335, 1966.
39. Tissieres, A., Watson, J. D., Schlessinger, D., and Hollingsworth, R. B. Ribonucleoprotein particles from *Escherichia coli*. *J. Molec. Biol.* **1**:221-233, 1959.
40. Rodgers, A. Magnesium ions and structure of *E. coli* ribosomal ribonucleic acid. *Biochem. J.* **100**:102-109, 1966.
41. Tissieres, A., and Watson, J. D. Ribonucleoprotein particles from *Escherichia coli*. *Nature* (London) **182**:778-780, 1958.
42. Chao, F. C. Dissociation of macromolecular ribonucleoprotein of yeast. *Arch. Biochem.* **70**:426-434, 1957.
43. Tso, P. O. P., Benzer, S., and Vinograd, J. Microsomal nucleoprotein particles from pea seedlings. *J. Biophys. & Biochem. Cytol.* **2**:451-465, 1956.
44. [illegible] Properties of microsomal nucleoprotein particles from pea seedlings. *Biochim. et Biophys. acta* **33**:[illegible], 1958.
45. Hamilton, M. G., and Petermann, M. L. Ultracentrifugal studies of ribonucleoprotein from rat liver microsomes. *J. Biol. Chem.* **234**:1441-1446, 1959.
46. Edelman, I. S., Tso, P. O. P., and Vinograd, J. Binding of [illegible] nesium to microsomal nucleoprotein and ribonucleic acid. *Biochim. et Biophys. acta* **42**:393-403, 1960.
47. Gros, F., et al. Unstable ribonucleic acid revealed by pulse labeling of *Escherichia coli*. *Nature* (London) **190**:581-585, 1961.
48. Brenner, S., Jacob, F., and Meselson, M. Unstable messenger RNA carrying information from genes to ribosomes for protein synthesis. *Nature* (London) **190**:576-581, 1961.
49. Cannon, M., Krug, R., and Gilbert, W. Binding of [illegible] *Escherichia coli* ribosomes. *J. Molec. Biol.* **7**:360-372, 1963.
50. *Enzymatic Synthesis of DNA*. Edited by A. Kornberg. New York: Wiley, 1962. *Ciba Foundation Symposium*, Pp. [illegible]
51. Lehman, I. R. Deoxyribonucleases of Escherichia coli purification and properties of phosphodiesterase. *J. Biol. Chem.* **235**:[illegible], 1960.
52. Hoagland, M. B., Stephenson, M. L., Scott, J. F., Hecht, L. I., and Zamecnik, P. C. Soluble ribonucleic acid intermediate in protein synthesis. *J. Biol. Chem.* **231**:241-252, 1958.
53. Matthaei, J. H., and Nirenberg, M. W. Characteristics and stabilization of DNAase-sensitive protein synthesis in *E. coli* extract. *Proc. Nat. Acad. Sci.* **47**:1880-1888, 1961.
54. Hoagland, M. B., Keller, E. B., and Zamecnik, P. C. Enzymatic carboxyl activation of amino acids. *J. Biol. Chem.* **218**:[illegible], 1956.
55. Schweet, R. S., and Allen, E. H. Purification and properties of tyrosine activating enzyme of beef pancreas. *J. Biol. Chem.* **232**:1099-1105, 1958.
56. Webster, L. T., Jr., and Davie, E. W. Purification and properties of serine-activating enzyme from beef pancreas. *J. Biol. Chem.* **235**:479-484, 1960.
57. Novelli, G. D. Amino acid activation for protein synthesis. *Ann. Rev. Biochem.* **36**:449-464, 1967.
58. Allende, J. E., Allende, C. C., Gianca, M., and Maldonado, M. Formation of threonyl adenylate enzyme complex. *Biochim. et Biophys. Research Commun.* **16**:342-346, 1964.
59. Nemes, A. T., and Berg, P. Mechanism of aminoacyl RNA synthesis: studies with isolated aminoacyl adenylate complexes of tyrosyl RNA synthetase. *Proc. Nat. Acad. Sci.* **52**:393-[illegible], 1964.
60. Lagerkvist, U., and Waldenstrom, J. Some properties of tRNA valyl and isoleucyl ribonucleic acid synthetases from yeast. *J. Biol. Chem.* **240**:2264, 1965.
61. Allende, J. E., Mora, G., Gianca, M., and Allende, C. C. Effect of metal ions on formation of threonyl adenylate intermediate from threonyl adenylate enzyme complex. *J. Biol. Chem.* **240**:PC 3229-PC 3232, 1965.
62. Lindahl, L., Adams, A., and Fresco, J. R. Renaturation of transfer ribonucleic acids through site binding of magnesium. *Proc. Nat. Acad. Sci.* **55**:941-948, 1966.
63. Williams, R. J. P., and Wacker, W. E. C. Cation balance in biological systems. *J. A.M.A.* **201**:18-22, 1967.
64. *The Pharmacological Basis of Therapeutics*. Third edition. Edited by L. S. Goodman and A. Gilman. New York: Macmillan, 1965.
65. Katz, B., and Miledi, R. Timing of calcium action during neuromuscular transmission. *J. Physiol.* **189**:543-548, 1967.
66. Feldberg, W. Anesthesia and sleep-like conditions produced by injections into cerebral ventricles of cats. *J. Physiol.* **140**:[illegible], 1958.
67. Katz, B., and Miledi, R. Study of spontaneous miniature potentials in spinal motoneurones. *J. Physiol.* **168**:389-422, 1963.
68. Sonjen, G., Hilay, M., and Stephen, C. R. Failure to cause [illegible] human subjects by intravenous administration of magnesium sulfate. *J. Pharmacol. & Exper. Therap.* **154**:652-659, 1966.
69. Peck, C. H., and Meltzer, S. J. Anesthesia in human beings by intravenous injection of magnesium sulfate. *J.A.M.A.* **67**:1131-1136, 1916.
70. Kruse, H. D., Orent, E. R., and McCollum, E. V. Studies on magnesium deficiency in animals. I. Symptomatology resulting from magnesium deprivation. *J. Biol. Chem.* **53**:519-539, 1932.
71. Wang, R., and Welt, L. G. Observations in experimental magnesium depletion. *J. Clin. Investigation* **42**:405-413, 1963.
72. Heggtveit, H. A., Herman, F., and Medina, R. E. Cardiac [illegible] and calcification in experimental magnesium deficiency: [illegible] electron microscopic study. *Am. J. Path.* **45**:797-[illegible], 1964.
73. Goldsmith, R. A. Relative magnesium deficiency in rats. *J. Nutrition* **93**:87-102, 1967.
74. MacIntyre, I., and Davidson, D. Production of secondary potassium depletion, sodium retention, nephrocalcinosis and hypercalciuria by magnesium deficiency. *Br. J. [illegible]*, 1966.

75. Martindale, L., and Heaton, F. W. Magnesium deficiency in adult rat. *Biochem. J.* 92:119-126, 1964.
76. Mitsuda, H., Hellerstein, E. E., Nakamura, M., and Lown, B. Effects of magnesium deficiency on diet upon puppies. *Circulation Research* 9:387-392, 1961.
77. Mulay, A., Velez, H., Guzman, C., and Cortes, P. Magnesium deficiency in man. *Circulation Research* 12:643-650, 1963.
78. Smith, R. H. Absorption of magnesium in large intestine of calf. *Nature* 174 (Supp.): 1119-1121, 1954.
79. Hirsch, C. [illegible] Hypomagnesemia in calves. 3. Endogenous fecal excretion of Ca, Mg, and P. *Biochem. J.* 71:306-311, 1959.
80. Smith, R. H. Importance of magnesium in control of plasma calcium in calf. *Nature* 181:161-162, 1958.
81. McCance, R. A., and Widdowson, E. M. Composition of food. *Med. Research Council Special Report* (London) 297:1960.
82. Seelig, M. S. Requirement of magnesium by normal adult: summary and analysis of published data. *Am. J. Clin. Nutrition* 14:242-290, 1964.
83. Spollema, B. Nutritional and metabolic disorder in cattle. *Nutrition Abstr. & Rev.* 1:621-632, 1932.
84. Allcroft, R. Hypomagnesemia, summary of evidence. *Agric. Rev.* (London) 1:1-4, 1955.
85. *Idem.* Hypomagnesemia of cattle and sheep in Britain. *J. Brit. Grassl. Soc.* 2:119, 1956.
86. Dishington, I. W. Changes in serum magnesium levels of ruminants, as influenced by diet effect of oral administration of various inorganic and organic compounds on serum magnesium level. *Acta Vet. Scandinav.* 6:159-177, 1965.
87. McSheese, D. M., and Forbes, R. M. Experimental production of magnesium deficiency in lambs on diet containing sorghage. *Nature* (London) (Supp.) 261:2025, 1959.

SEMINARS IN MEDICINE

OF THE

BETH ISRAEL HOSPITAL, BOSTON

DAVID S. FEIGOLD, M.D., *Editor*
EDITH E. PARRIS, *Assistant Editor*

RELATIVE IMPORTANCE OF PARATHYROID HORMONE AND THYROCALCITONIN IN CALCIUM HOMEOSTASIS*

LOUIS M. SHERWOOD, M.D.

ALTHOUGH it has long been recognized that the serum calcium is maintained within rigid physiologic limits, it is only in the past decade that the hormonal factors involved in this regulation have been defined in biochemical terms and that detailed investigation of their mode of action has been initiated. Developments during this period have included the following: purification of bovine parathyroid hormone (PTH) and partial characterization of the molecular features essential for its biologic activity[1]; examination of a variety of in vivo and in vitro models to define the mechanism of action of the hormone[2], direct measurement of PTH in bovine serum by radioimmunoassay, permitting detailed investigation of the physiology of the parathyroid gland[3]; and discovery of a new hormone, thyrocalcitonin (TCT),[4,5] which has an action antagonistic to that of PTH and whose properties are being investigated in many laboratories. Since an objective of these seminars is to relate advances in basic science to human biology, I shall review these recent developments and consider their possible relevance to clinical disorders of calcium homeostasis. Although the roles of PTH and TCT will be emphasized, it is appreciated that thyroxin, growth hormone, adrenal corticosteroids and estrogen may also contribute to calcium homeostasis.

After thyroparathyroidectomy in man, the serum calcium falls to about 6 mg per 100 ml and remains in constant and dynamic equilibrium with a labile portion of the skeleton.[6] The precise biochemical and enzymatic factors involved in the equilibrium between extracellular mineral and bone crystal are still unclear, but only a small portion of the 1 kg of calcium in the skeleton is in contact with the interstitial fluid. The site of equilibrium is presumably the surface of bone, an extensive area where many local factors may be operating. In addition to mineral, the stable portion of the skeleton contains a complex organic matrix composed of collagen, mucopolysaccharides and cellular elements; parathyroid excess leads to breakdown of these organic elements as well as the mineral matrix. McLean[6] suggested that the parathyroid glands exert an additional control on calcium homeostasis by mobilizing calcium from stable portions of bone and raising serum calcium to the normal range of 9 to 11 mg per 100 ml.

PARATHYROID HORMONE

Although the parathyroid glands were first described about 100 years ago, their physiologic significance was not appreciated until the early 1900's, when the tetany that frequently followed thyroidectomy was ascribed to the inadvertent removal of the parathyroid tissue. Collip,[7] in 1925, made the first active preparation of PTH, and during the next two decades, Albright and his colleagues[8] elucidated much of what is now known about the physiology of the hormone as well as the clinical disorders of calcium metabolism.

A new era of research began in 1959, when Aurbach[9] prepared stable, partially purified hormone by

*From the Department of Medicine, Columbia University College of Physicians and Surgeons, New York, New York 10032.

Supported by a training grant (T1-AM5397-05) from the United States Public Health Service, an institutional grant (IN-54-L) from the American Cancer Society, and a grant (AM09579) from the National Institutes [illegible]

Yale J. Biol. + Med., 5: 545-553, 1933

THE EFFECT OF MAGNESIUM SULPHATE ON THE BRAIN OF THE FETAL RAT*

F. A. WIES

The cause or causes of monstrosities have intrigued observers for many years. Many attempts to explain the origin of these beings have given rise to the wildest speculation. The curse of the gods, sexual congress with lower animals, or lunar influences, as is indicated by the very word "monster", meaning "moon-calf" were for a long time universally accepted causes. The most tenacious, and apparently well-supported notion of causality, specified the factor of maternal impression. The typical example, recurring with endless variations, is that of the pregnant mother being frightened by a frog hopping out from under a bush, and subsequently giving birth to a fetus resembling, more or less closely, a frog.

That the appearance of monsters is not just an occasional happening but a very frequent event is emphasized by Mall[7], who states that seven out of every hundred pregnancies among human beings result in pathological embryos, many of which are aborted. This author, from experiments upon a variety of vertebrates, as well as from the study of pathological human ova, is of the opinion that the power to become a monster is present in every ovum.

In recent years the problems of teratology have been attacked by the experimental method. This method, together with certain embryological and clinical facts, has disclosed two chief factors operative in maldevelopment as in normal development, namely, germinal and environmental. The evidence permits of no escape from the fact that many variations, anomalies, and malformations are hereditary. Harelip, albinism, and polydactylism are examples of this class. On the other hand, there is abundant evidence to indicate that untoward environmental conditions, including nutritive wants and vitamin deficiencies, are highly productive of monstrosities and the whole range of anomalous developmental results.

Development is the result of the interaction between the inherent tendencies contained within the egg substance itself and the

---

* Based upon a thesis submitted to the Faculty of the Yale University School of Medicine in candidacy for the Degree of Doctor of Medicine. June, 1933. From the Department of Anatomy.

external conditions or environment. The usual interaction of these factors gives rise to normal animal forms. When, however, either factor is changed, an unusual form results. Various degrees of imperfection in either or both result in endless degrees of variations, anomalies, malformations, and monstrosities.

An intermediate step in the chain of causation may be a disturbance of function or balance in the endocrine system. Imbalance between the thyroid gland and the hypophysis, producing conditions of dwarfism and gigantism, is an example of this kind.

The untoward environmental factors leading to the development of monstrosities are various. For the most part they may be classified as mechanical, physiochemical, nutritional, and conditions causing faulty implantation or developmental arrest at critical stages. It is obvious that the line of demarcation between these groups of factors is not always sharp.

Among the mechanical factors which affect development adversely, amniotic bands take a leading part. These bands arise from the originally solid primordium of the amniotic cavity, and are especially prevalent in the carnivora. They may become wrapped about the legs or arms, or even about the neck. In extreme circumstances amputation may be produced.

In 1884, Schultze[13] produced frog monsters by placing the eggs between sheets of glass and then inverting them. Spemann[14] (1903), working with the same species, produced monsters by tying pieces of fine thread around the developing eggs. Lewis[5] (1909) caused cyclopeia in *Fundulus heteroclitus* embryos by pricking the anterior end of the embryonic shield with a needle. The rudiments were thus brought into contact and fused.

It is well known that large doses of X-rays adversely affect the fetus. This has been demonstrated by experiments in animals and has been found to occur after treatment of the pregnant mother as a form of therapy. Kaplan[4] (1932) reports the case of an hydrocephalic monster with distorted features whose mother had had X-ray therapy to the ovaries in the early part of pregnancy.

Extensive experimentation in lower forms has contributed a large body of data bearing on the teratological effects of slight variation in the optimum physiochemical conditions under which development normally proceeds.

In 1903, Morgan[11] produced spina bifida in frogs by subjecting the eggs to the action of common salt solution. A 0.6 per cent

solution delayed the process of gastrulation for from 12 to 24 hours. After this delay spina bifida resulted, the exposed cord later undergoing cytolysis.

The best-known experiments of this kind are those of Stockard[15] (1909) who showed that by treating the eggs of *Fundulus heteroclitus* with solutions of magnesium salts cyclopeian monsters could be produced at will. In 1929, Musselman[12] reported the effect of the addition of saline or alcohol to chicken eggs 16 hours after the beginning of incubation. In from 48 to 72 hours later there was noted a failure of the head folds to close or an absence of the pituitary gland, the eyes, or the olfactory pits.

Such alterations in the physiochemical milieu of the developing germ may be related to variations occurring under more normal or favorable nutritional conditions. Under nutritional factors of teratogenesis may be listed reductions in the normal oxygen supply, lack of food, and vitamin deficiencies. The introduction into the body of the mother of alcohol, narcotics, industrial poisons like lead, certain drugs in the case of unsuccessful attempts at abortion, and the "autotoxins" of imperfect digestion and excretion may be of significance.

Mall[7] has emphasized faulty implantation of the ovum as a teratological factor. He noted that early-aborted terata were commonly associated with uterine inflammation, frequently the result of gonorrheal or syphilitic infection, which prevented proper imbedding of the egg. However, Corner's studies[1] (1923) of defective ova and the associated uterine mucosa in the pig suggest that endometritis may have been overemphasized as a factor in human teratogenesis. He has shown that in the pig about ten per cent degenerate after becoming blastocytes, and about five to ten per cent become abnormal during the subsequent course of pregnancy. This leaves only about seventy per cent to develop into normal, viable pigs. Since the uterine mucosa overlying these degenerate ova was both histologically and functionally normal, Corner concludes that in the pig at least, delayed or faulty implantation is not a causative factor of monstrosities.

Stockard[16] (1921) has emphasized possibly the commonest factor of malformation, the factor of developmental arrest at critical stages. A given animal species passes through its embryonic stages at a specific rate of development, probably dependent upon the rate of oxidation in the protoplasm of the species. Should variation in

this rate exceed certain limits, the developmental result frequently becomes modified or distorted. The rate of development is not uniform throughout the entire process, but periods of rapid progress alternate with movements of slow rate or almost quiescence. Such a discontinuous mode of development is universal among the birds and is known to occur in several mammals, notably the armadillo. Working with the salt-water minnow Stockard interrupted development just before gastrulation by lowering the temperature or reducing the oxygen supply. After a brief period, development was reinstituted by allowing the temperature and available oxygen to return to normal. By this method various grades of duplicate monstrosities could be produced. The explanation offered for this effect is that at certain critical stages of development, particularly the beginning of gastrulation, normal development demands that a certain point in the blastoderm, the point of infolding of the primitive streak, have developmental precedence over all other points. If development be stopped by some means, other points in the blastoderm have the same advantage in the matter of developmental leadership when growth starts again with the return of favorable conditions. Thus opportunity is given for more than one point to develop, for example two or more primitive streaks; various types and degrees of fission and fusion then result.

Wilder[17] (1908) tried to show that abnormal forms are due to the development of abnormal or unusual germs. He arranged certain types of monsters in a "cosmobiotic" series. He argued that the regular and orderly manner in which members of this series develop indicates that such abnormal forms are due directly to some abnormal variation within the germ cells from which they arose, instead of any unusual external influence upon the eggs after development had started. However, Stockard[15] (1909) described just such a cosmobiotic series which he produced artificially. Beginning with a cyclopeian monster having one median eye we pass through a scale of individuals with the median eye separating into two approximated eyes, then to those with normally-placed eyes, then to those with two heads and three eyes, and finally we reach a two-headed monster with four eyes.

Burr has attempted to integrate the seemingly inexplicable and unrelated facts in ontology in the following hypothesis: "The fundamental constitution of a biologic system is determined by a

characteristic bioelectric field which organizes, regulates, and integrates the growth and differentiation of all the component parts of the entire organism operating through protoplasm as its expression". From this point of view any factor which changed the electrodynamic field of a biological system might be expected to be reflected in a change in the development of makeup of the organism.

The experiments of Morgan[11], Stockard[15], and Musselman[12], described above, have indicated what a profound rôle alterations in the physiochemical environment may have in the developing individual. These investigators used species of animals relatively low in the vertebrate scale, like the frog or Fundulus, in which the developing embryo could be directly observed and its environment directly altered at will.

The possibility of causing anomalies of development in the central nervous system of the young of mammals by indirectly changing their environmental milieu occurred to the writer. That the nervous system is extremely sensitive to alterations of the biochemical environment was well shown by Gildea and Cobb[3] (1930) in their experiments on the effects of anemia on the cerebral cortex of the cat. They demonstrated that obstruction of the blood supply to the brain for periods of ten minutes or more results in areas of focal necrosis in the cortex, most marked in laminae III and V. The pyramidal cells were especially susceptible.

Obviously, in species of animals whose definitive development takes place in the uterus of the mother, the milieu could be affected only indirectly, *i.e.*, by altering the physiology of the mother. Certain of such alterations could change intrauterine conditions and so be indirectly reflected in the fetus.

It was decided to attempt to effect such an alteration by injecting pregnant rats with a solution of magnesium sulphate. From the alimentary canal the rate of absorption for this drug is too slow to produce systemic effects, but if injected parenterally, it acts as a powerful depressor of the central and peripheral nervous systems. When administered intravenously in rabbits until the concentration in the blood reached 0.1 per cent, McGuigan[8] (1928) found a complete loss of excitability of the central nervous system and a curare-like paralysis of the motor nerves to striated muscles. According to Meltzer[9] (1916) the inhibitory properties of magnesium sulphate

are due to the fact that it enters with ease into the lymph bathing the synaptic membranes, and thus interrupts the passage of nervous impulses from one neuron to the other.

Mendel and Benedict[10] (1909) found that doses of magnesium sulphate up to 0.8 g. per kilo were not fatal in dogs. Subcutaneous administration never caused abscess formation or necrosis when this form of magnesium was used. They found that the greater part of the drug left the body by way of the kidneys within 48 hours. Excretion by way of the intestine was of minor significance. Measurable quantities might be retained in the body for periods of at least two weeks. Parenteral administration in dogs and rabbits was never followed by purgation.

In this series of experiments 18 female and 16 male albino rats were used. Their ages ranged from about 120 to 200 days while under observation. Rats during this period of life may be expected to be sexually active and fertile. Long and Evans[6] (1922) find the first œstrus to occur on the average on the seventy-sixth day and the first copulation about ten days later. Three sets of experiments were performed, one in the spring, one in the fall, and one in the winter of 1932.

A sterile solution of magnesium sulphate was administered subcutaneously to the females every other day in doses of about 0.8 g. $MgSO_4.7H_2O$ per kilo. The dosage was varied slightly depending on the susceptibility of the individual animal.

A typical protocol, that for Rat F I, follows:

3-7-32. Weight 180 g. Put in separate cage with M I.

3-9-32. Wt. 185 g. 1 cc. 25% Magnesium sulphate subcutaneously in back at 3:30 P.M. 3:35 looks slightly limp, turns over and lies on side. 4:00 still on side; reacts to touch and sound. Corneal reflex positive. 4:10 on belly. 4:25 drinking. 4:30 completely recovered.

3-11-32. Wt. 190 g. 3:50, 0.5 cc. subcutaneously. 4:00 no effect. M I taken away.

3-13-32. Wt. 190 g. 10:47, 0.6 cc. subcutaneously. 11:00 very slight weakness. 11:05 on side. 11:07 on belly; breathing rapid and deep. 11:25 recovered.

3-15-32. Wt. 185 g. 9:25, 0.7 cc. subcutaneously. 9:35 slightly limp. 9:40 on side; breathing rapid. 9:45 on belly. 9:50 again on side. 9:51 on belly; breathing slower. 10:05 recovered, drinking.

3-16-32. Looks sick; actions very sluggish.

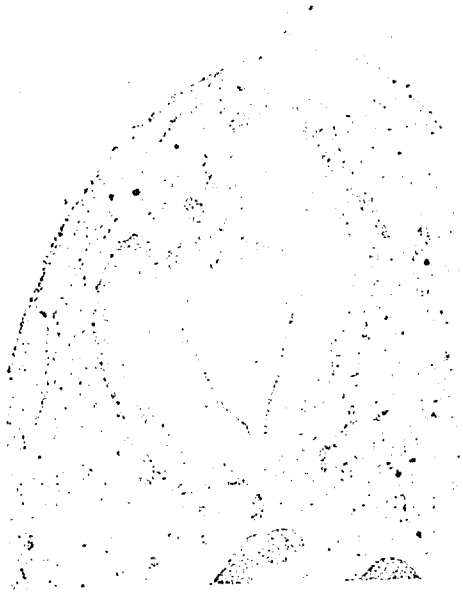


Fig. I

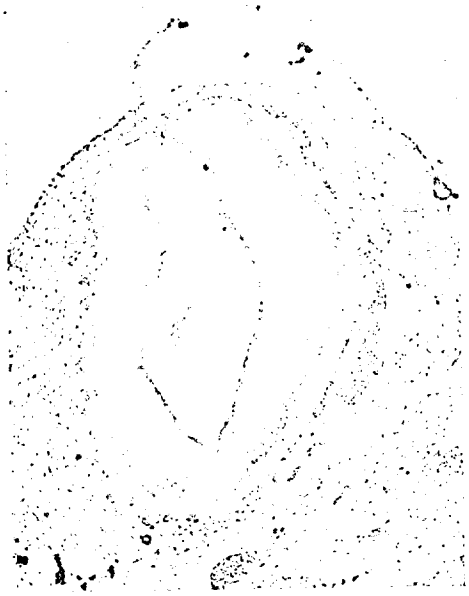


Fig. II

3-17-32. Looks well. Wt. 200 g. 2:10, 0.7 cc. subcutaneously. 2:20 slightly weak; on side, breathing rapid; able to crawl. 2:25 occasional convulsive movements of whole body. 2:27 crawling on belly. 2:35 on side, very limp. 2:40 on belly, grinding teeth. 3:20 recovered.

3-19-32. . Wt. 197 g. 10:45, 0.6 cc. subcutaneously. 9:26 started to jump around. 9:28 respirations stopped. Immediate autopsy. The uterus was large and divided into eight lobes, each containing a fetus. Over the surfaces of the lobes were small winding vessels of a purple color. One lobe was placed in Bouin's solution and later sectioned. The other organs appeared normal. The needle paths were explored and found to be subcutaneous. There was no abscess formation in the surrounding tissue.

The females were watched carefully to detect possible abortion. No aborted fetuses were found, and none of the uteri which were examined gave evidence of having expelled products of conception.

A fetus from F XIV, a rat which had received no injections, measured 4.9 mm. in its long axis. Judging from the studies of Donaldson[2], this fetus would be about 11 days old. Complete serial sections showed this fetus to be normal in all respects.

The fetus from F I, the rat whose protocol is given above, was estimated to be about ten days old. In the region of the midbrain, extending through 15 serial sections, a defect is noticed. This defect is quite evident under low-power magnification, and gives the appearance of a bite taken out of the wall of the neural tube. Figure I is a photomicrograph with a magnification of 75 diameters of this area. High-power magnification shows that the defect is due to a lack of neuroblasts in the mantle layer. The spongioblasts are normal in this area, making the marginal layer appear thicker than usual. This appearance is due entirely to lack of neuroblasts, however. There are fewer germinal cells undergoing mitosis in the ependymal layer bordering this defect than in the ependymal layer elsewhere. The capillaries in this region, as well as elsewhere, are filled with nucleated red blood cells. There is no infiltration of white blood cells or fixed tissue cells into this area.

Along the greater part of the spinal cord the lateral walls have failed to fuse, to close over the central canal. There is thus no roof plate in this region. This condition begins at the cephalad end of the cord and extends caudalward for 168 serial sections, or to about the middle of the embryo. Here the canal is closed over by a very thin roof plate. This extends for 70 serial sections, but is lacking thereafter till the very end of the cord is reached. Figure II is a photomicrograph of this condition at the cephalad end of the

cord. As far as could be determined the cells constituting the various layers of the cord, *i.e.*, the ependymal, the mantle, and the marginal layers, are within normal limits. There is no evidence of an inflammatory change. All the other tissues are normal.

The fetuses from two other rats which had received injections and had become pregnant were normal in all respects, both to gross and to microscopic observation.

Before the actual experimental work was begun it was expected that some of the rats would abort as a result of the injections. However, later careful observation of the females proved this not to be the case, and microscopic examination of the uteri revealed no evidence of expelled products of conception.

Perhaps the most striking result was the low fertility resulting from matings of the females receiving the magnesium sulphate. Only three out of twenty such matings, or 15 per cent resulted in pregnancies. On the other hand, both of two females which did not receive the drug and which were used to secure control embryos became pregnant very shortly after mating. The low fertility of the injected females can be explained only on the basis of the injections. All other conditions were conducive to fertility. The males and females were all healthy, of breeding age, adequately fed and housed, kept at an equable temperature, and disturbed as little as possible. The œstrus cycle averages five days, and in this experiment the couples were kept together for periods of three weeks. The fact that fifteen of the matings occurred in the fall months played some rôle, since it is well known that rats are less fertile in the fall than in the spring. However, this could by no means account for a fertility rate of only 15 per cent. Under the conditions of the experiment we are left with only one other factor which could be of importance, the injections of magnesium sulphate.

Marked individual differences were noted in the degree of narcosis produced by the doses of magnesium sulphate used, and two of the animals succumbed to not unusually large doses. In one of the animals marked convulsions preceded exitus, and death in both instances was a respiratory one. Anesthesia and motor paralysis preceded the stoppage of respiration.

The total amount of the drug received by some of the animals was very large. One animal received 4.2 gms. over a period of

eight weeks. Yet, outside of the intervals immediately following injections, it remained alert and active and gained 25 grams in weight. In spite of two matings, this animal did not become pregnant.

It is realized that the defects noted in the brain and spinal cord are only suggestive. To be classed as positive results these would have to be duplicated in a series of animals, allowing some of them to reach maturity if possible, and watching them for any functional disturbances of behavior. That the defects were developmental arrests and not traumatic lesions due to the direct effect of the magnesium sulphate ions seems fairly certain. There is absolutely no evidence of attempts at repair on the part of the surrounding tissues. There are no leukocytic or fixed tissue responses and no fibrotic changes. All that is noted in the midbrain is a circumscribed area showing diminution in the number of cells of the neuroblastic series. In the cord there is a lack of a roof plate, leaving the spinal canal open.

References

1 Corner, G.: Am. J. Anat., 1923, *31*, 523.
2 Donaldson, H.: The Rat. Memoir Wistar Inst., Philadelphia, 1925.
3 Gildea, E. F., and Cobb, S.: Arch. Neurol. & Psychiat., 1930, *23*, 876.
4 Kaplan, I.: Am. J. Gyn. & Obs., 1932, *23*, 426.
5 Lewis, W.: Anat. Rec., 1909, *3*, 175.
6 Long, J., and Evans, H.: Memoirs of the Univ. of Calif., 1922, *6*.
7 Mall, F. P.: J. Morphol., 1908, *19*, 1.
8 McGuigan, H.: Textbook of Pharmacology and Therapeutics. W. B. Saunders Co., 1928.
9 Meltzer, S. J.: J. Am. Med. Asso., 1916, *66*, 1931.
10 Mendel, L. B., and Benedict, S. R.: Am. J. Physiol., 1909, *25*, 1.
11 Morgan, T. H.: Arch. f. Entwicklungsmech. d. Organ., 1903, *16*, 691.
12 Musselman, L. K.: Proc. Soc. Exper. Biol. & Med., 1929, *27*, 216.
13 Schultze, O.: Arch. f. Entwicklungsmech. d. Organ., 1894, *1*, 269.
14 Spemann, H.: Arch. f. Entwicklungsmech. d. Organ., 1903, *16*, 551.
15 Stockard, C. R.: J. Exper. Zool., 1909, *6*, 285.
16 Stockard, C. R.: Am. J. Obs., 1909, *59*, 582.
17 Wilder, H.: Am. J. Anat., 1908, *8*, 355.

Arch. Toxikol.
27:129-135, 1971.

Toxicologic-chemical findings in a fatal magnesium poisoning following duodenal administration of magnesium sulfate*

by

G. Willner

From: "Carl Gustav Carus" Medical Academy, Dresden, Institute of Forensic Medicine.

Sent in: Sept. 17, 1970.

Translation of German summary:

On the basis of a fatal case of poisoning following an intraduodenal overdose of magnesium sulfate, quantitative magnesium determinations were carried out for the first time in various body fluids and different segments of the gastro-intestinal tract. The values obtained are discussed in comparison with isolated findings previously reported in the literature.

Key words: Magnesium Sulfate - Accidental Lethal Poisoning - Colorimetric Molybdenum Blue Method - Distribution in the Organism.

Magnesium sulfate (magnesium sulfuricum), $MgSO_4$ or Epsom salts, is used therapeutically in various concentrations and forms of application.

---

* Dedicated to Prof. W. Reinmann, M.D. on his 50th birthday

According to Hauschild (1961) when administered per os in isotonic solutions (10 to 30 g in 1/4 to 3/4 liter water) it serves as a purgative. In higher concentrations magnesium sulfate is used for duodenal flushing. Thus for instance Heilmeyer and Gitter (1965) recommend a 15% magnesium sulfate solution (20-50 ml) in cholecystopathies, while according to Hauschild (1961) as a rule 10 to 40% solutions (30-100 ml) are used for duodenal flushing. Since in high doses magnesium sulfate has a vermifugal effect, 70-100 g (intraduodenal) serves as an anthelmintic according to Ludewig and Lohs (1966). Hauschild (1961) recommends a dose of 80-100 g through a duodenal tube in cases of tapeworm infestation.

According to reports from the DAB (German Pharmacopeia) 7, (1964), magnesium sulfate is used in a 10 to 25% solution for intramuscular injections in tetanus or eclampsia. Twenty-five or 50% magnesium sulfate injection solutions can be used in anesthesia - as an additive - to reduce the necessary quantity of ether.

With magnesium concentrations of 10 mg % in the blood, according to Hauschild and Görisch (1967), there is somnolence, while with a concentration of 15-20 mg% a completely reflexless anesthesia sets in. As a rule 28-35 mg% leads to death from paralysis of the respiratory centers.

Although magnesium sulfate is generally considered a relatively slightly toxic substance, a number of fatalities have nevertheless been described in the literature.

So far as is known these can all be attributed to its peroral and more particularly intraduodenal administration. In such cases relatively high doses were used in large concentrations.

According to Schumann (1961) in the older literature there are a total of 12 cases of poisoning following peroral ingestion of magnesium sulfate (designated as Epsom salts in the English-language literature), of which 8 ended in death. The quantities ingested ranged between 1 and 4 ounces (ca. 30-120 g). Dotzauer and Hornbostel in 1952 described a 56 year old man who died 4 hours after having magnesium sulfate administered to him for the first time by duodenal intubation (total 90 g); Rösler (1961) has reported the case of a 62 year old woman who died 7 hours after duodenal administration of magnesium sulfate (60 g).

Two fatalities resulting from tapeworm cures with magnesium sulfate have been described by Fossel (1952): a 63 year old woman received 200 ml 40% and 30 ml 30% $MgSO_4$ solution through a duodenal tube, that is to say a total of 89 g magnesium sulfate. Death took place 8 hours later. The magnesium content of the blood was more than three times the normal (not specified) values. In the second case a 46 year old woman with heart disease drank 250 ml concentrated magnesium sulfate solution (ca. 70 g). Death took place after 3 days.

A detailed description of a fatal case of magnesium poisoning in the course of a routine tapeworm cure was published by

Schumann in 1961. A 68 year old woman, in addition to 0.8 g atebrin, received a total of 100 g magnesium sulfate as purgative. The substance was administered through a duodenal tube in two lots of 100 ml of a 50% solution at 45 minute intervals. Death took place 3 hours later from acute heart failure.

In 1962 the same author reported another similar case of magnesium sulfate poisoning. Here again after the administration of 0.8 g atebrin and 230 ml of a 30% magnesium sulfate solution (69 g) through a duodenal tube, death took place ca. 5 1/2 hours later from acute heart failure.

The first data on toxicologic-chemical findings in fatal magnesium sulfate poisoning are to be found in exactly comparable and confirmed form in the work of Schumann (1961, 1962). The post mortal serum magnesium levels determined by him by the complexometric method were 19.5 and 19.7 mg% in the case of the 68 year old woman, while in the case of the 67 year old woman the post mortal magnesium level in the blood was 40 mg%.

Since the normal intravital magnesium content of the serum or whole blood in humans, according to concordant data in the literature, is ca. 2-3 mg% or 2-4 mg% (e.g. Hauschild, 1961; Hauschild and Görisch, 1967; Hoppe-Seyler/Thierfelder, 1953; Schumann, 1961; in Schummann, further literature), while on the other hand it is known that after death considerable ionic changes take place and 24-48 hours post mortem there is generally a rise to ca. 7 mg% magnesium, Schumann (1961) only regards magnesium values over 15 mg% measured after death as

a manifestation of an increased intravital magnesium content of the blood.

There is a study by Paulus (1962) on the magnesium content in the cadaver urine after ingestion of magnesium sulfate. In a fatal case following the peroral administration of large quantities of 30% magnesium sulfate solution he determined a urinary value of 100 mg% magnesium by the titanium yellow method. His comparative studies on normal cadaver urine yielded values of ca. 1-4 mg% magnesium, while according to Hoppe-Seyler/Thierfelder (1953) values up to ca. 20-30 mg% magnesium may be taken as the intravital normal range in the urine.

A report will be given below on a fatality following intraduodenal administration of an overdose of magnesium sulfate, which, due to exact knowledge of the previous history, was made the subject of a specific toxicologic-chemical study and thus allowed quantitative magnesium determinations in several material samples.

History: A 59 year old woman was admitted to the hospital after several days of illness with violent vomiting and body pains. The diagnosis was: "Obstructive jaundice and gallbladder hydrops with suspicion of cholelithiasis, subileus".

After the obstructive syndrome had subsided, a cholangiography was carried out.

In the course of the diagnostic tests the patient was

mistakenly given 400 (!) ml of a 40% magnesium sulfate solution by duodenal tube. This was immediately followed by severe body pains, after five minutes there was circulatory collapse, the patient was no longer responsive. Despite appropriate therapeutic measures with administration of calcium, fluids, treatment of the circulation and artificial respiration, death took place ca. 6 hours later.

The autopsy was performed the following day.

Toxicologic-chemical analyses

Material

Immediately after the autopsy blood samples (heart blood, blood from a right leg vein), the stomach contents (ca. 170 ml light brown, aqueous fluid), tied-off segments with fluid from the duodenum, the duodenojejunal flexure, the median segment of the small intestine, the last flexure of the ileum and the ascending colon, the remaining contents of the small intestine, the right and left vitreous fluid (each ca 1 ml), urine (100 ml) and parts of the brain, liver and kidneys were made available for toxicologic-chemical analyses.

In addition, guaranteed samples from one bottle with the remaining quantity of magnesium sulfate slution still in existence (2.6 ml) and a second bottle, said to contain the same magnesium sulfate solution (ca. 370 ml) were available.

Methods

The identification and purity tests (guaranteed samples) were made according to the specifications of German Pharmacopeia

7 (1964). The magnesium content of the two samples (pure magnesium sulfate solutions) was determined by complexometry with eriochrome black T as indicator.

The quantitative determination of the magnesium content of the various body fluids and fluid components from the gastro-intestinal tract took place colorimetrically according to the method of Fretwurst-Maennchen (1953); separation of calcium oxalate - precipitation as magnesium ammonium sulfphate - conversion into complex phosphomolybdenic acid - reduction to molybdenum blue. The samples were prepared in a manner analogous to the usual method for serum, and to confirm the findings, appropriate test solutions and comparative samples were tested simultaneously.

Results

1. Samples.

Identification and purity tests of the two solutions: no objections raised.

Magnesium sulfate content ($MgSO_4.7H_2O$):

Remains of solution (2.6 ml) 41.0 weight %

Solution from the same batch (370 ml) 38.0 weight %.

2. Autopsy material.

Qualitative tests for magnesium ions in the fluid samples from the gastrointestinal tract and in the urine: strongly positive reactions.

Magnesium content in mg% (in brackets: quantity of material

examined):

Heart blood (10 ml)	33.20
Blood from right leg vein (10 ml)	33.21
Urine (100 ml)	90.50
Right vitreous (ca. 1 ml)	9.96
Left vitreous (ca. 1 ml)	7.97
Stomach contents (170 ml)	31.54
Duodenum (5 ml)	10.21
Duodenojejunal flexure (60 ml)	26.14
Median segment of small intestine (70 ml)	24.15
Last flexure of ileum (5 ml)	52.29
Ascending colon (290 ml)	6.80
Rest of contents of small intestine (330 ml)	18.59

The following substances or groups of substances were excluded as other possible sources of poisoning: volatile poisons, oxydizing substances, toxic metal and arsenic compounds, strong pharmaceutical products, alkaloid poisons, CO- and Met-Hb, reducing blood components.

Discussion

In the present case it was possible for the first time, by specific toxicologic-chemical analyses, to determine the magnesium content of several body fluids and the magnesium concentration in different segments of the gastrointestinal tract in a fatal case following the intraduodenal administration of a large quantity of magnesium sulfate.

By comparison with similar fatalities caused by magnesium sulfate, a quantitative concordance is observed between the present chemical findings and data obtained from the literature.

Schumann (1961, 1962) in 2 cases (100 g $MgSO_4$, death after 3 hours; 69 g $MgSO_4$, death after ca. 5 1/2 hours) found magnesium levels of ca. 20 mg% and c. 40 mg% in the blood; the value obtained in our case (160 g $MgSO_4$, death after c. 6 hours) in the heart blood and the blood from a leg vein was c. 33 mg% magnesium. This finding , obtained after a roughly 1 1/2 to twice higher dose of magnesium sulfate, would seem to confirm the view of Schumann (1962) that magnesium values measured in the blood after death from 15 mg% up indicate a toxic intravital magnesium content.

The urine value in a fatality following the peroral ingestion of large quantities of 30% magnesium sulfate solution was determined by Paulus (1962) as ca. 90 mg%.

No data could be obtained on the average magnesium content of the human vitrous fluid. On the other hand the following values were reported by Hoppe-Seyler/Thierfelder (1953) for some animal species: horse 1.7 mg%, cow 2 mg%, hog 3 mg% magnesium. The inorganic substance content of the aqueous humor is believed to be strongly dependent upon age and to accord fairly well with the serum value of the same individual. Based on the latter finding and the magnesium concentration of some animal vitreous fluids that lie roughly within the normal

range of human serum it could be concluded that the values of ca. 8-10 mg% magnesium established in the present case were probably 2 to 3 times higher than the normal magnesium content of the human vitreous fluid. The magnesium concentrations established in the different segments of the gastrointestinal tract parallel with the magnesium content of various body fluids naturally scarcely allow conclusions to be drawn as to the degree of the magnesium poisoning, but in our view they can be used as reference values of possible quantities in doubtful cases of poisoning of a similar nature with a fatal outcome.

Bibliography

1. DAB 7: German Pharmacopeia, 7th Edition. Berlin, Akademie Verlag, 1964.

2. Dotzauer, G., and Hornbostel, H.: The use of magnesium sulfate from the standpoint of 'Nil nocere'. Ärztl. Wschr. 7:1102, 1952.

3. Fossel, M.: Fatalities after peroral administration of magnesium sulfate. Beitr. gerichtl. Med. 19:39, 1952.

4. Fretwurst, F., and Maennchen, K.: Photometric methods in medical chemistry. Wetzlar: Komm. Verlag Scharfes Druckereien KG, 1953.

5. Hauschild, F.: Pharmacology and foundations of toxicology, Third edition, Leipzig, VEB Georg Thieme, 1961.

6. - and Görisch, V.: Introduction to pharmacology and prescriptions. Second edition. Leipzig: VEB Georg Thieme, 1967.

7. Heilmeyer, L. and Gitter, A.: Pocket prescription book. 14th edition, Jena: VEB Gustav Fischer, 1965.

8. Hoppe-Seyler/Thierfelder: Handbook of physiological and patho-chemical analysis. 10th edition, Berlin-Göttingen-Heidelberg: Springer, 1953.

9. Ludewig, R., and Lohs, K.H.: Acute poisoning. Jena: VEB Gustav Fischer, 1966.

10. Paulus, W.: The magnesium content of cadaver urine after ingestion of large quantities of magnesium sulfate. Arch. Toxikol. 19:325, 1962.

11. Rösler, F. Cited by H.J. Schumann, Münch. med. Wschr. 103:1865, 1961.

12. Schumann, H.-J.: Nil nocere! Fatal magnesium poisoning following correctly conducted tapeworm treatment. Münch. med. Wschr. 103:1865, 1961.

13. - : Nil nocere!Fatal magnesium poisoning following a correctly conducted tapeworm treatment. II. Münch. med. Wschr. 105:2463, 1962.

Author's address: G. Willner, "Carl Gustav Carus" Medical Academy,
Institute of Forensic Medicine
Fetscherstrasse 74
8019 Dresden
East Germany.

Arch. Toxikol. 27, 129—135 (1971)
© by Springer-Verlag 1971

Toxikologisch-chemische Befunde bei einer tödlichen Magnesiumvergiftung nach duodenaler Verabfolgung von Magnesiumsulfat*

G. Willner

Medizinische Akademie „Carl Gustav Carus" Dresden
Institut für Gerichtliche Medizin

Eingegangen am 17. September 1970

Toxicologic-Chemical Findings in a Lethal Magnesium Poisoning Following Duodenal Administration of Magnesium Sulfate

Abstract. In a case of accidental lethal poisoning whereby a 59 year old woman undergoing cholangiography received by mistake 400 ml of a 40% magnesium sulfate solution into the duodenum and died 6 hours later, quantitative determinations of magnesium in various body fluids and at different levels of the gastrointestinal tract were carried out for the first time.

The magnesium concentrations obtained in mg percent using the colorimetric (Molybdenum blue) method were as follows: Heart blood = 33.20 (10 ml); blood from a vein in the right leg = 33.21 (10 ml); urine = 90.50 (100 ml); vitreous humor of the right eye = 9.96 (approx. 1 ml); vitreous humor of the left eye = 7.97 (approx. 1 ml); stomach contents = 31.54 (170 ml); duodenum = 10.21 (5 ml); duodenojejunal flexure = 26.14 (60 ml); middle region of the small intestine = 24.15 (70 ml); last portion of ileum = 52.29 (5 ml); ascending colon = 6.80 (290 ml); and the remaining contents of the small intestine = 18.59 (330 ml).

The values obtained were compared with individual findings previously reported in the literature and the data discussed.

Key-Words: Magnesium Sulfate — Accidental Lethal Poisoning — Colorimetric Molybdenum Blue Method — Distribution in the Organism.

Zusammenfassung. Anhand eines tödlichen Vergiftungsfalles nach intraduodenaler Überdosierung von Magnesiumsulfat werden erstmals in verschiedenen Körperflüssigkeiten und einzelnen Abschnitten des Magen-Darm-Traktes quantitative Magnesiumbestimmungen durchgeführt und die ermittelten Werte unter Berücksichtigung der bisher aus der Literatur bekannten Einzelbefunde diskutiert.

Schlüsselwörter: Magnesiumsulfat — Accidentelle tödliche Vergiftung — Kolorimetrische Molybdänblau-Methode — Verteilung im Organismus.

Magnesiumsulfat (Magnesium sulfuricum, offiz.) $MgSO_4 \cdot 7\,H_2O$, Bittersalz findet in unterschiedlichen Applikationsformen und Konzentrationen therapeutische Anwendung.

* Herrn Prof. Dr. sc. med. W. Reinmann zum 50. Geburtstag gewidmet.

Per os dient es nach Hauschild (1961) in isotonischer Lösung (10 bis 30 g in $^1/_4$—$^3/_4$ Liter Wasser) als Abführmittel. In höheren Konzentrationen wird Magnesiumsulfat u.a. für Duodenalspülungen angewandt. So empfehlen z. B. Heilmeyer und Gitter (1965) eine 15%ige Magnesiumsulfatlösung (20—50 ml) bei Cholecystopathien, nach Hauschild (1961) werden für Duodenalspülungen allgemein 10- bis 40%ige Lösungen (30—100 ml) benutzt. Da Magnesiumsulfat in hohen Dosen vermifug wirkt, dienen nach Ludewig und Lohs (1966) 70—100 g (intraduodenal) als Anthelminthicum, Hauschild (1961) empfiehlt, bei Täniose eine Dosis von 80—100 g zweckmäßig mittels Duodenalsonde zu geben.

Als 10- oder 25%ige Injektionslösung wird Magnesiumsulfat nach Angaben des DAB 7 (1964) intramuskulär bei Tetanus und Eklampsie verabfolgt. 25- oder 50%ige Magnesiumsulfat-Injektionslösungen können bei Narkosen — zusätzlich verabfolgt — zur Herabsetzung der notwendigen Äthermenge verwendet werden.

Bei einer Magnesiumkonzentration im Blut von 10 mg-% besteht nach Hauschild und Görisch (1967) Schläfrigkeit, bei 15—20 mg-% tritt volle reflexlose Narkose ein. 28—35 mg-% führen meist infolge Lähmung des Atemzentrums zum Tode.

Obwohl Magnesiumsulfat im allgemeinen als eine verhältnismäßig wenig toxische Substanz gilt, ist dennoch in der Literatur eine Reihe von Todesfällen beschrieben worden.

Diese sind sämtlich — soweit bekannt — auf seine Anwendung bei peroraler, insbesondere jedoch bei intraduodenaler Verabfolgung zurückzuführen. Hierbei wurden jeweils relativ große Dosen in hohen Konzentrationen angewandt.

Nach Schumann (1961) finden sich in der älteren Literatur insgesamt 12 Vergiftungsfälle nach peroraler Einnahme von Magnesiumsulfat (im englischsprachigen Schrifttum als Epsomsalz bezeichnet), von denen 8 tödlich verliefen. Die eingenommenen Mengen lagen zwischen 1 und 4 Unzen (etwa 30—120 g) Epsomsalz. Dotzauer und Hornbostel berichten 1952 von einem 56jährigen Mann, der 4 Std nach erstmalig mittels Duodenalsonde verabfolgtem Magnesiumsulfat (insgesamt 90 g) starb; Rösler (1961) teilt den Fall einer 62jährigen Frau mit, die 7 Std nach duodenaler Magnesiumsulfat-Gabe (60 g) ad exitum kam.

2 Todesfälle bei Bandwurmkuren mittels Magnesiumsulfat beschreibt Fossel (1952): Eine 63jährige Frau erhielt duodenal 200 ml 40%ige und 30 ml 30%ige $MgSO_4$-Lösung, d.h. insgesamt 89 g Magnesiumsulfat. Der Tod trat 8 Std später ein, der Magnesiumgehalt des Blutes betrug mehr als das Dreifache der (nicht genannten) Normalwerte. Im zweiten Fall trank eine 46jährige herzkranke Frau insgesamt 250 ml konzentrierte Magnesiumsulfatlösung (ca. 70 g), Exitus nach 3 Tagen.

Eine ausführliche Darstellung einer tödlichen Magnesiumvergiftung bei regelrecht durchgeführter Bandwurmkur erfolgte 1961 durch Schumann. Eine 68jährige Frau erhielt neben 0,8 g Atebrin insgesamt 100 g Magnesiumsulfat als Abführmittel. Die Zuführung erfolgte mittels Duodenalsonde in zweimaliger Gabe von je 100 ml einer 50%igen Lösung im Abstand von 45 min. Exitus letalis 3 Std später durch akutes Herzversagen.

Vom gleichen Autor wird 1962 über eine weitere ähnliche Magnesiumsulfatvergiftung berichtet. Auch hier trat nach Gaben von 0,8 g Atebrin und 230 ml einer 30%igen Magnesiumsulfatlösung (69 g) per Duodenalsonde der Tod ca. $5^1/_2$ Std später unter akutem Herzversagen ein.

Angaben über bei tödlichen Magnesiumsulfat-Vergiftungen erhobene toxikologisch-chemische Einzelbefunde finden sich in exakt vergleichbarer und entsprechend gesicherter Form erstmalig bei Schumann (1961, 1962). Die von ihm nach der komplexometrischen Methode ermittelten postmortalen Serum-Magnesium-Spiegel ergaben im Fall der 68jährigen Frau Werte von 19,5 und 19,7 mg-% Magnesium, im Fall der 67jährigen Frau lag der postmortal ermittelte Magnesiumspiegel im Blut bei 40 mg-%.

Da der normale intravitale Magnesiumgehalt des Serums bzw. Gesamtblutes beim Menschen nach übereinstimmenden Literaturangaben etwa 2—3 mg-% bzw. 2—4 mg-% beträgt (z. B. Hauschild, 1961; Hauschild u. Görisch, 1967; Hoppe-Seyler/Thierfelder, 1953; Schumann, 1961; dort weitere Literaturangaben), andererseits bekannt ist, daß sich nach dem Tode erhebliche Ionenverschiebungen einstellen und 24—48 Std post mortem im allgemeinen eine Erhöhung auf etwa 7 mg-% Magnesium eintritt, wertet Schumann (1961) erst postmortal gemessene Magnesiumwerte über 15 mg-% als Ausdruck eines erhöhten intravitalen Magnesiumgehaltes im Blut.

Hinsichtlich des Magnesiumgehaltes im Leichenurin nach Aufnahme von Magnesiumsulfat liegt eine Untersuchung von Paulus (1962) vor. Er konnte in einem Todesfall nach peroraler Aufnahme größerer Mengen 30%iger Magnesiumsulfatlösung mittels Titangelbmethode einen Urinwert von 100 mg-% Magnesium bestimmen. Nach seinen vergleichenden Untersuchungen normaler Leichenurine ergaben sich Werte von etwa 1—4 mg-% Magnesium, nach Hoppe-Seyler/Thierfelder (1953) kann man als intravitalen Normalbereich im Urin Werte bis etwa 20—30 mg-% Magnesium annehmen.

Im folgenden soll über einen Todesfall nach intraduodenaler Verabfolgung einer Überdosis Magnesiumsulfat berichtet werden, der infolge genau bekannter Vorgeschichte gezielt zur toxikologisch-chemischen Untersuchung kam und deshalb quantitative Magnesiumbestimmungen in mehreren Materialproben ermöglichte.

Zum Vorgang: Eine 59jährige Frau wurde nach mehrtägiger Erkrankung mit heftigem Erbrechen und Leibschmerzen im Krankenhaus aufgenommen. Die vorläufige klinische Diagnose lautete: „Verschlußikterus und Gallenblasenhydrops bei Verdacht auf Cholelithiasis, Subileus."

Nach Abklingen des Verschlußsyndroms war u.a. eine Cholangiographie vorgesehen.

Im Rahmen der diagnostischen Erhebungen wurden der Patientin mittels Duodenalsonde versehentlich 400 (!) ml einer 40%igen Magnesiumsulfatlösung verabfolgt. Danach traten sofort heftige Leibschmerzen auf, nach 5 min kam es zum Kreislaufkollaps, die Patientin war nicht mehr ansprechbar. Trotz entsprechender therapeutischer Maßnahmen mit Gaben von Calcium, Flüssigkeitszufuhr, Kreislaufbehandlung und künstlicher Beatmung trat ca. 6 Std später der Tod ein.

Die Sektion erfolgte am nächsten Tag.

Toxikologisch-chemische Untersuchungen

Material. Unmittelbar post sectionem wurden Blutproben (Herzblut, Blut aus der rechten Beinvene), Mageninhalt (ca. 170 ml hellbraune, wäßrige Flüssigkeit), abgebundene Teile mit Flüssigkeit des Duodenums, der Flexura duodeno-jejunalis, des mittleren Dünndarmabschnittes, der letzten Ileumflexur und des Colon ascendens, der restliche Dünndarminhalt, Glaskörperflüssigkeit rechts und links (je ca. 1 ml), Urin (100 ml) sowie Teile von Gehirn, Leber und Niere zur toxikologisch-chemischen Untersuchung übergeben.

Ferner waren an sichergestellten Asservaten eine Flasche mit der noch vorhandenen Restmenge Magnesiumsulfatlösung (2,6 ml) sowie eine zweite Flasche, die die gleiche Magnesiumsulfatlösung enthalten sollte (ca. 370 ml), vorhanden.

Methoden. Die Identitäts- und Reinheitsprüfungen (sichergestellte Asservate) erfolgten nach den Vorschriften des DAB 7 (1964). Der Magnesiumgehalt der beiden Asservatproben (reine Magnesiumsulfatlösungen) wurde komplexometrisch mit Eriochromschwarz T als Indicator ermittelt.

Die quantitative Bestimmung des Magnesiumgehaltes der verschiedenen Körperflüssigkeiten und flüssigen Anteile aus dem Magen-Darm-Trakt erfolgte colorimetrisch nach der Methode von Fretwurst-Maennchen (1953): Calciumoxalatabtrennung — Fällung als Magnesiumammoniumphosphat — Überführung in komplexe Phosphormolybdänsäure — Reduktion zu Molybdänblau. Die Probenaufarbeitung geschah analog der üblichen Methodik für Serum, zur Sicherung der Befunde wurden entsprechende Testlösungen und Vergleichsproben mitgeführt.

Ergebnisse

1. Asservate

Identitäts- und Reinheitsprüfung beider Lösungen: ohne Beanstandungen.

Magnesiumsulfatgehalt ($MgSO_4 \cdot 7\,H_2O$):

noch vorhandene Restlösung (2,6 ml)	41,0 Gew.-%
Lösung gleicher Charge (370 ml)	38,0 Gew.-%

2. Sektionsmaterial

Qualitative Prüfungen auf Magnesium-Ionen in den Flüssigkeitsproben des Magen-Darm-Traktes und im Urin: stark positive Reaktionen.

Magnesiumgehalt in mg-% (in Klammer: vorhandene Menge Untersuchungsmaterial)

Herzblut (10 ml)	33,20
Blut aus rechter Beinvene (10 ml)	33,21
Urin (100 ml)	90,50
Glaskörperflüssigkeit rechts (ca. 1 ml)	9,96
Glaskörperflüssigkeit links (ca. 1 ml)	7,97
Mageninhalt (170 ml)	31,54
Duodenum (5 ml)	10,21
Flexura duodeno-jejunalis (60 ml)	26,14
mittlerer Dünndarmabschnitt (70 ml)	24,15
letzte Ileumflexur (5 ml)	52,29
Colon ascendens (290 ml)	6,80
restlicher Dünndarminhalt (330 ml)	18,59

Der Ausschluß evtl. anderer chemischer Intoxikationsquellen erfolgte für folgende Substanzen bzw. Stoffgruppen: flüchtige Gifte, oxydierend wirkende Stoffe, toxische Metall- und Arsenverbindungen, stark wirkende Pharmaka, Alkaloidgifte, CO- und Met-Hb, reduzierende Blutbestandteile.

Diskussion

Im vorliegenden Fall konnten erstmals bei einem Todesfall nach intraduodenaler Verabfolgung einer großen Menge Magnesiumsulfat durch gezielte toxikologisch-chemische Untersuchungen der Magnesiumgehalt mehrerer Körperflüssigkeiten und die in einzelnen Abschnitten des Magen-Darm-Traktes vorhandenen Magnesiumkonzentrationen ermittelt werden.

Bei einer vergleichenden Gegenüberstellung der in etwa sich ähnelnden Todesfälle durch Magnesiumsulfat kann man eine größenordnungsmäßige Übereinstimmung der vorliegenden chemischen Untersuchungsbefunde mit den in der Literatur enthaltenen Angaben feststellen.

Schumann (1961, 1962) fand in 2 Fällen (100 g $MgSO_4$, Exitus nach 3 Std; 69 g $MgSO_4$, Exitus nach ca. $5^1/_2$ Std) Magnesiumspiegel im Blut von ca. 20 mg-% bzw. ca. 40 mg-%; die in unserem Fall (160 g $MgSO_4$, Exitus nach ca. 6 Std) im Herz- bzw. Beinvenenblut erhaltenen Werte betragen ca. 33 mg-% Magnesium. Dieser nach einer etwa $1^1/_2$- bis 2fach höheren Dosis Magnesiumsulfat erhaltene Befund dürfte die von Schumann (1962) vertretene Ansicht bestätigen, daß postmortal ge-

messene Magnesiumwerte im Blut ab 15 mg-% auf einen toxischen intravitalen Magnesiumgehalt schließen lassen.

Der Urinwert bei einem Todesfall nach peroraler Aufnahme größerer Mengen 30%iger Magnesiumsulfat-Lösung wurde von Paulus (1962) mit 100 mg-% bestimmt, der in unserem Fall festgestellte Magnesiumgehalt im Leichenurin beträgt ca. 90 mg-%.

Über den durchschnittlichen Magnesiumgehalt der menschlichen Glaskörperflüssigkeit konnten keine Angaben erhalten werden. Dagegen finden sich bei Hoppe-Seyler/Thierfelder (1953) für einige Tierarten folgende Werte: Pferd 1,7 mg-%, Rind 2 mg-%, Schwein 3 mg-% Magnesium. Der Gehalt der Kammerflüssigkeit an anorganischen Bestandteilen soll stark vom Lebensalter abhängen und beim gleichen Individium verhältnismäßig gut mit dem Serumwert übereinstimmen. Auf Grund des letzteren Hinweises und der etwa im Normalbereich menschlichen Serums liegenden Magnesiumkonzentrationen einiger tierischer Glaskörperflüssigkeiten könnte gefolgert werden, daß die im vorliegenden Fall ermittelten Werte von ca. 8—10 mg-% Magnesium gegenüber dem normalen Magnesiumgehalt der menschlichen Glaskörperflüssigkeit um wenigstens das Doppelte bis Dreifache erhöht sein dürften. Die in den einzelnen Abschnitten des Magen-Darm-Traktes parallel zum Magnesiumgehalt verschiedener Körperflüssigkeiten ermittelten Magnesiumkonzentrationen lassen selbstverständlich kaum eine Aussage über den Grad einer Magnesium-Intoxikation zu, sie sind jedoch nach unserer Auffassung geeignet, bei fraglichen ähnlichen Vergiftungsfällen mit tödlichem Ausgang wenigstens als Vergleichswerte einer möglichen Größenordnung zu dienen.

Literatur

DAB 7: Deutsches Arzneibuch, 7. Ausgabe. Berlin: Akademie-Verlag 1964.

Dotzauer, G., Hornbostel, H.: Anwendung von Magnesiumsulfat unter dem Gesichtspunkt des Nil nocere. Ärztl. Wschr. 7, 1102 (1952).

Fossel, M.: Todesfälle nach peroraler Verabreichung von Magnesiumsulfat. Beitr. gerichtl. Med. 19, 39 (1952).

Fretwurst, F., Maennchen, K.: Photometrische Methoden in der Medizinischen Chemie. Wetzlar: Komm. Verlag Scharfes Druckereien KG 1953.

Hauschild, F.: Pharmakologie und Grundlagen der Toxikologie, 3. Aufl. Leipzig: VEB Georg Thieme 1961.

— Görisch, V.: Einführung in die Pharmakologie und Arzneiverordnungslehre, 2. Aufl. Leipzig: VEB Georg Thieme 1967.

Heilmeyer, L., Gitter, A.: Rezepttaschenbuch, 14. Aufl. Jena: VEB Gustav Fischer 1965.

Hoppe-Seyler/Thierfelder: Handbuch der physiologisch- und pathologisch-chemischen Analyse, 10. Aufl. Berlin-Göttingen-Heidelberg: Springer 1953.

Ludewig, R., Lohs, K. H.: Akute Vergiftungen. Jena: VEB Gustav Fischer 1966.

Paulus, W.: Der Magnesiumgehalt im Leichenurin nach Aufnahme größerer Mengen von Magnesiumsulfat. Arch. Toxikol. 19, 325 (1962).

Rösler, F.: Zit. nach H.-J. Schumann, Münch. med. Wschr. 103, 1865 (1961).

Schumann, H.-J.: Nil nocere! Tödliche Magnesium-Vergiftung bei regelrecht durchgeführter Bandwurmkur. Münch. med. Wschr. 103, 1865 (1961).

— Nil nocere! Tödliche Magnesium-Vergiftung bei regelrecht durchgeführter Bandwurmkur. II. Münch. med. Wschr. 105, 2463 (1962).

Dipl.-Chem. G. Willner
Medizinische Akademie
„Carl Gustav Carus"
Institut für Gerichtliche Medizin
DDR-8019 Dresden
Fetscherstr. 74
Deutschland

Proc. Soc. Exp. Biol. Med. 25: 587

Conclusion. Combinations of amidopyrin with magnesium chlorid in experimental animals exhibit antipyretic synergism, and, to a lesser extent, diminished toxicity.

3965

Influence of Magnesium Salts Upon the Toxicity and Antipyretic Action of the Salicylates.

J. E. WINTER AND H. G. BARBOUR.

From the Department of Physiology and Pharmacology, School of Medicine, University of Louisville, Kentucky.

In the preceding paper we have shown that magnesium chlorid is antipyretic to rabbits in hay-infusion fever. Furthermore the antipyretic efficiency of amidopyrin was found considerably potentiated by combination with magnesium chlorid, although in the same combination the toxicity appears slightly decreased.

The synergistic action of magnesium combined with antipyretic drugs is further illustrated by the following experiments upon magnesium combinations with salicylates. The work will be described under 3 heads: (1) toxicity in normal mice of sodium salicylate alone and in combination with $MgCl_2$; (2) antipyretic action in fevered rabbits of sodium salicylate alone and combined with $MgCl_2$; (3) antipyretic action in fevered dogs of aspirin alone and in combination with magnesium oxide.

1. *Toxicity.*

In normal white mice, the m.l.d. (minimal lethal dose) of sodium salicylate was found to be about 600 mg. per kilo (Table I). Similarly, the m.l.d. of a combination of 2 parts sodium salicylate with 3 parts magnesium chlorid (anhydrous) was found to be approximately 975 mg. per kilo (Table II). Our criterion for judging the m.l.d., as will be seen, does not include an occasional fatality within that range of dosage where survival is obviously the rule.

In the previous report, it was shown that the m.l.d. for magnesium chlorid is approximately 1.05 gm. per kilo. Thus the predicted m.l.d. for the present combination with sodium salicylate would be 807.5* mg. per kilo. Inasmuch as 975 mg. were, with 1 exception, actually required, there is evidently an antagonism between the 2 drugs favorable to survival. The combination is about

* For prediction formula see other paper.

TABLE I. *Toxicity in Normal White Mice.*

Drug*	Mg. per kilo	Number of mice	
		Survived	Died
Sodium salicylate	550	5	1
	600	6	2
	650	2	2
	700	0	1
	1,000	0	2
	1,200	0	2
	1,400	0	2
	1,600	0	2
Sodium salicylate + $MgCl_2$ (2:3)	875	3	0
	900	5	1
	925	3	0
	975	0	2
	1,025	0	2
	1,050	2	1

* 1% solutions.

20% less toxic than predicted. This accords with the mutual antagonism shown between many depressant drugs when combined, and is most readily explained by the wider distribution of the *loci* of toxic action.

2. *Antipyretic Action in Rabbits.*

In a series of 11 rabbits, the average temperature reduction resulting from the subcutaneous injection of sodium salicylate (in 5% solution) in doses of 100 mg. per kilo, amounted to 0.7° F. in 2 hours, and 1.0° in 4 hours. These effects are illustrated in Fig. 1, where the average curve is compared with the antipyretic curves resulting from 2 different mixtures of sodium salicylate (5%) and magnesium chlorid (5%).

The above amount of sodium salicylate was combined in 8 rabbits with magnesium chloride 150 mg. per kilo (ineffective alone). The temperature fell so rapidly that the former 2-hour level of 0.7° F. was attained during the first half hour. Sodium salicylate alone had practically no effect within that period. At the end of 2 hours, the average temperature was still about half a degree below that produced by the salicylate alone, but by the fourth hour, the magnesium effect wearing off, the temperature had begun to return to normal so that the position of the 2 curves nearly coincided.

Even when only 50 mg. per kilo of sodium salicylate were given with magnesium chlorid 150 mg. per kilo rapid reduction in temperature was noted. This combination was found more efficient in 13 rabbits, than 100 mg. per kilo sodium salicylate alone, for a period of over one and one-half hours. By the fourth hour, however, the temperature had returned practically to the original febrile level.

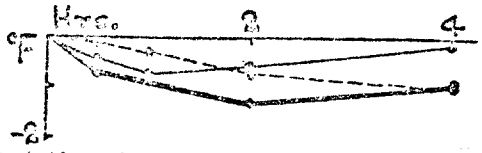


FIG. 1. Effects in fevered rabbits of subcutaneous administration of sodium salicylate alone and with magnesium chlorid, upon body temperature.

Abscissae, time after administration of antipyretic.

Ordinates, temperature changes °F., not absolute but calculated as difference from control fevered rabbits. Seventeen fever controls.

Dosage per kilo: Broken line, Na salicylate 100 mg. (average of 11 rabbits). Light continuous line, Na salicylate 50 mg. plus $MgCl_2$ 150 mg. (av. 13 rabbits). Heavy continuous line, Na Salicylate 100 mg. plus $MgCl_2$ 150 mg. (av. 8 rabbits).

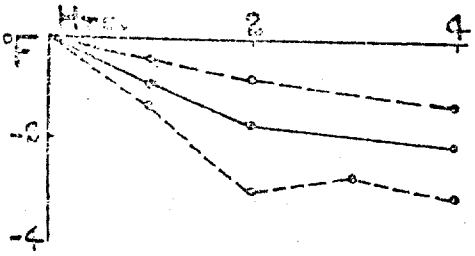


FIG. 2. Effects in fevered dogs of oral administration of aspirin alone and with magnesium chlorid. No fever controls. The temperature decreases are absolute rather than relative.

Dosage per kilo: Upper broken line, aspirin 100 mg. (1 dog). Lower broken line, aspirin 200 mg. (av. 2 dogs). Continuous line, aspirin 100 mg. plus $MgCl_2$ 150 mg. (av. 4 dogs).

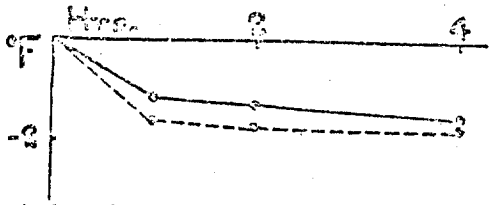


FIG. 3. Effects in fevered dogs of oral administration of aspirin alone and with magnesium oxid. Four fever controls.

Dosage per kilo: Broken line, aspirin 150 mg. (av. 4 dogs). Continuous line, aspirin 100 mg. plus MgO 100 mg. (av. 5 dogs).

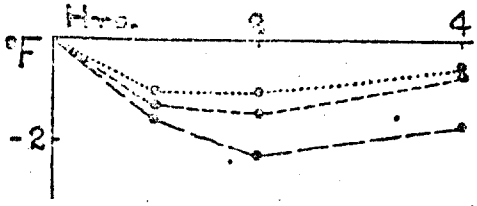


FIG. 4. Effects in fevered dogs of oral administration of magnesium oxid. One fever control.

Dosage per kilo: Dotted line, MgO 75 mg. (1 dog). Short dash line, MgO 150 mg. (1 dog). Long dash line, MgO 300 mg. (1 dog).

The slow, long-continued temperature fall produced by salicylate is thus shown to be modified to a more rapid though briefer effect when magnesium chlorid is added. It is significant that amounts of magnesium chlorid below the antipyretic threshold are sufficient to cause such a modification even when the salicylate dose is cut in half. Thus marked synergism outlasts the first hour. The crossing of the curves after a certain time is analogous to that noted with amidopyrin-magnesium antipyresis and definitely indicates that magnesium salts potentiate the early effects of antipyretic drugs, even when intense or prolonged action tends to be inhibited.

3. *Antipyretic Action of Aspirin and Magnesium by Mouth in Dogs.*

Aspirin administered by mouth† to fasting, fevered dogs in doses of 100 and 200 mg. per kilo, gave respective decreases in temperature of 1.3° (1 dog) and 3.1° F. (2 dogs) at the end of 4 hours (Fig. 2). The curve of antipyresis resulting from a combination of aspirin 100 mg. per kilo plus magnesium chlorid 150 mg. per kilo (4 dogs), occupies an almost exactly intermediate position. The combination thus accomplished the same result as would require about 150 mg. per kilo of aspirin alone.

Further experimental series were performed, using magnesium oxide in heavy suspension instead of the chlorid. In substituting magnesium oxide for magnesium chlorid, the dose used was reduced by one-third, giving a slightly higher molecular concentration of magnesium. As seen in Fig. 3, this combination (100 mg. of each drug) was not as effective as 150 mg. per kilo of aspirin alone, although presumably better than would be obtained by 100 mg. per kilo. The effect of the combination for the first 2 hours was slightly better than that produced by 150 mg. per kilo magnesium oxid alone, and did not show the remission seen 4 hours after the latter. (See Fig. 4, which shows the effects of 3 different doses of magnesium oxide.)

The weaker action of magnesium oxide as compared with the chlorid may be ascribed to slower absorption.

Conclusions. 1. Magnesium salts in mice appear to reduce the toxicity of salicylates (protective antagonism).

2. Magnesium augments the antipyretic action of sodium salicylate and of aspirin. When given subcutaneously with salicylate to fevered rabbits, the earlier stages of antipyresis are characterized by marked synergism.

J. Pharm & Exp Therap. Vol 38: 1931

MAGNESIUM OXIDE AS AN AID TO THE ANTIPYRETIC ACTION OF PHENACETIN IN DOGS

J. E. WINTER, C. H. RICHEY AND H. G. BARBOUR

From the Department of Physiology and Pharmacology, School of Medicine University of Louisville, Kentucky[1]

Received for publication January 27, 1930

Recent papers from this laboratory have indicated that magnesium exerts a definite synergic effect when given in combination with certain antipyretics, e.g.: sodium salicylate (1), aspirin (1), cinchophen (3), amidopyrine (2). Such combinations have also shown uniformly a lower toxicity (judged from minimal lethal doses) than would be predicted from the known toxicity of their individual components. That such decreased toxicity can be accompanied by increased therapeutic effect of a combination of drugs is easily comprehensible. For two drugs may have a common therapeutic point of action but different toxic loci. Loewe has shown this with many combinations of hypnotic and antipyretic drugs, and obtained a striking example of hypnotic synergism and toxic antagonism by a combination of acetphenetidin, codeine and acetylsalicylic acid (4).

How dispersion of the locus of toxic action may be particularly beneficial in therapeutics, can be illustrated by the case of phenacetin. This analgesic is limited in its use by the fact that, like other anilin derivatives, it may interfere with oxygen transport by inducing methemoglobin formation. Given, however, with another analgesic substance not poisonous to the blood, the chance that analgesia will be accompanied by untoward effects upon the circulation becomes greatly reduced.

The following experiments were undertaken to ascertain whether or not the antipyretic effects of phenacetin in the dog

[1] In coöperation with the Pharmaceutical Division of the Calco Chemical Company, Bound Brook, New Jersey.

could be fortified substantially by simultaneous administration of magnesium oxide.

PROCEDURE

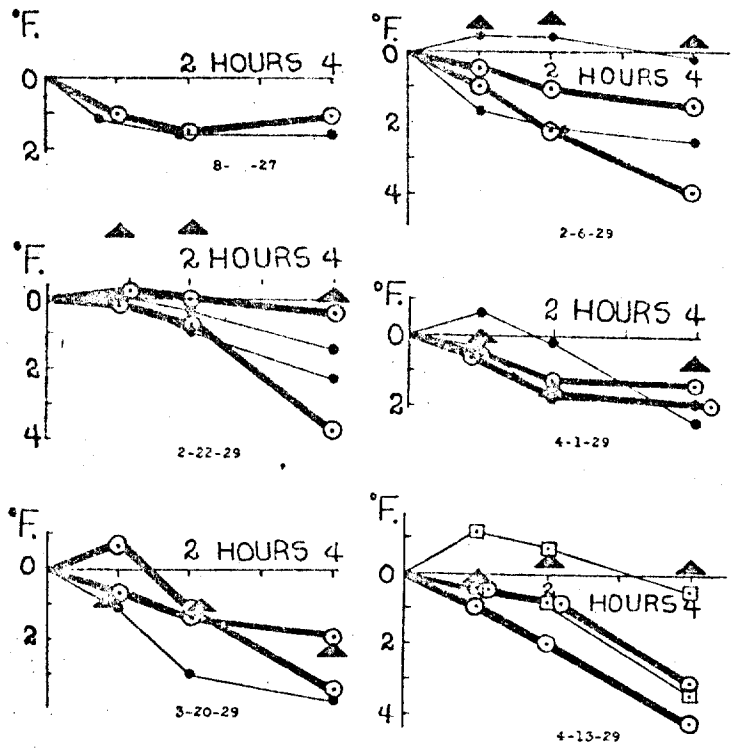
Only healthy adult dogs with medium or short hair were used. The method of inducing antipyresis has been previously detailed (3). All dogs were fasted two days. Two hours after injection of a number of dogs with fresh hay infusion, selection was made of those which exhibited increases in rectal temperature of more than a degree Fahrenheit. One of these was kept as fever control, while the rest were given, by stomach tube, either phenacetin or MgO or a combination of the two. One hundred cubic centimeters of water accompanied each administration. The rectal temperatures of the dogs were followed further at intervals of one, two and four hours after the drug administration.

RESULTS

The dose of the combination usually given was phenacetin 200 mgm. per kilogram plus MgO 100 mgm. per kilogram. In a preliminary experiment without fever control, the antipyretic effect was found quantitatively similar to that of phenacetin given alone in dosage of 300 mgm. per kilogram (fig. 1). The same doses were compared on four further experimental days, a fever control dog being used each time (figs. 2 to 5). In two of these (figs. 2 and 4) a distinct advantage was shown for the combination. In another (fig. 3) no essential difference was apparent while only on a day on which the fever control dog failed to maintain the usual high level of temperature (casting doubt upon the reliability of that day's hay infusion) did the uncombined phenacetin exhibit the greater antipyretic effect (fig. 5). In a further experiment a pair of dogs given the same combination was compared against a pair given only 200 mgm. per kilogram phenacetin. The average effect was decidedly in favor of the combination (fig. 6).

A further experiment on the effects of phenacetin (200 mgm. per kilogram) given alone again showed variability, one dog being more affected by it than dogs receiving twice as much phenacetin, while another showed hardly any effect.

When MgO by itself was administered to dogs in doses from 75 to 300 mgm. per kilogram, definite antipyresis resulted in 6 out of 15 cases (figs. 8 to 11). The variations noted were not related to the dose of MgO given. This makes a total of 9 cases



FIGS. 1-6. ANTIPYRETIC EFFECTS OF PHENACETIN 200 MGM. PER KILOGRAM PLUS MAGNESIUM 100 MGM. PER KILOGRAM (HEAVY LINES AND CIRCLES) COMPARED WITH PHENACETIN ALONE (LIGHT LINES)

Dots: 300 mgm. per kilogram; squares: 200 mgm. per kilogram; solid triangles: fever control dogs. Zero temperature is temperature at time of administration.

of antipyresis out of 20 experiments (cf. (1) and (3)) on oral administration of MgO in these doses.

Oral administration of antipyretic drugs to dogs, as the foregoing experiments show, inevitably yields variability of results. Only two series of dogs receiving the same dose are thought

extensive enough for the application of statistical methods. We gave phenacetin 300 mgm. per kilogram to 8 dogs and the combination of phenacetin 200 plus MgO 100 to 11 animals. The average results are plotted in figure 12, the ordinates here repre-

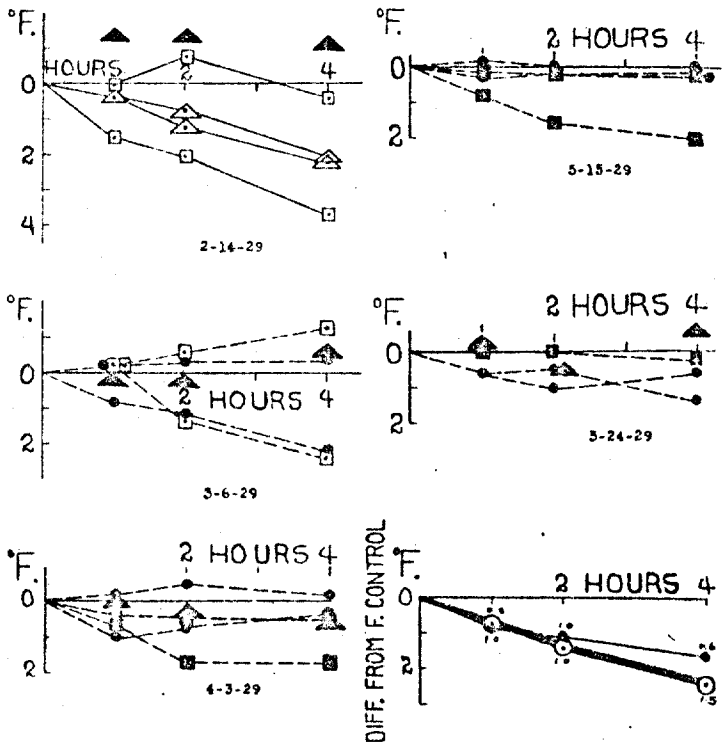


FIG. 7. ANTIPYRETIC EFFECTS OF PHENACETIN ALONE

Open triangles: 400 mgm. per kilogram; squares: 200 mgm. per kilogram; solid triangles: fever control dogs. Zero temperature is temperature at time of administration.

FIGS. 8–11. ANTIPYRETIC EFFECTS OF MgO

Solid squares: 75 mgm. per kilogram; open squares: 200 mgm. per kilogram; dots: 300 mgm. per kilogram; solid triangles: fever control dogs. Zero temperature is temperature at time of administration.

FIG. 12. AVERAGE CURVE OF 8 PHENACETIN RESULTS (LIGHT LINE) 300 MGM. PER KILOGRAM COMPARED WITH AVERAGE CURVE OF 11 PHENACETIN PLUS MgO RESULTS (HEAVY LINE) (200 PLUS 100 MGM. PER KILOGRAM)

Mean deviation recorded near each average point. Ordinates: temperature difference from fever control.

senting difference from the average fever control dog rather than absolute decrease in temperature. Near each average point plotted is recorded the mean deviation from this average. These curves show that phenacetin, 200 mgm. per kilogram, when given with MgO (100 mgm.) may be expected to exert at least as great an antipyretic effect as phenacetin, 300 mgm. per kilogram, given alone. Since doses of MgO even three times as large as that used in the combination, are not surely antipyretic we may regard our results as strongly indicative of true synergism.

CONCLUSION

Oral administration of phenacetin to dogs produces an antipyretic effect which is considerably enhanced by the addition of magnesium oxide. The results thus far obtained preclude final decision as to the presence or absence of synergism (i.e., potentiation as distinguished from simple summation); but they suggest trial of phenacetin fortified by magnesium in the hope of securing equal therapeutic benefit, with less chance of poisoning.

REFERENCES

(1) Winter, J. E., and Barbour, H. G.: Proc. Soc. Exper. Biol. and Med., 1928, xxv, 587.
(2) Barbour, H. G., and Winter, J. E.: Proc. Soc. Exper. Biol. and Med., 582.
(3) Barbour, H. G., and Winter, J. E: Jour. Pharmacol. and Exper. Therap., 1929, xxxv, 425.
(4) Loewe, S., Kaer, E., and Muischnek, H.: Arch. f. exper. Pathol. u. Pharmakol., 1927, cxx, 25.

eight weeks. Yet, outside of the intervals immediately following injections, it remained alert and active and gained 25 grams in weight. In spite of two matings, this animal did not become pregnant.

It is realized that the defects noted in the brain and spinal cord are only suggestive. To be classed as positive results these would have to be duplicated in a series of animals, allowing some of them to reach maturity if possible, and watching them for any functional disturbances of behavior. That the defects were developmental arrests and not traumatic lesions due to the direct effect of the magnesium sulphate ions seems fairly certain. There is absolutely no evidence of attempts at repair on the part of the surrounding tissues. There are no leukocytic or fixed tissue responses and no fibrotic changes. All that is noted in the midbrain is a circumscribed area showing diminution in the number of cells of the neuroblastic series. In the cord there is a lack of a roof plate, leaving the spinal canal open.

References

1 Corner, G.: Am. J. Anat., 1923, *31*, 523.
2 Donaldson, H.: The Rat. Memoir Wistar Inst., Philadelphia, 1925.
3 Gildea, E. F., and Cobb, S.: Arch. Neurol. & Psychiat., 1930, *23*, 876.
4 Kaplan, I.: Am. J. Gyn. & Obs., 1932, *23*, 426.
5 Lewis, W.: Anat. Rec., 1909, *3*, 175.
6 Long, J., and Evans, H.: Memoirs of the Univ. of Calif., 1922, *6*.
7 Mall, F. P.: J. Morphol., 1908, *19*, 1.
8 McGuigan, H.: Textbook of Pharmacology and Therapeutics. W. B. Saunders Co., 1928.
9 Meltzer, S. J.: J. Am. Med. Asso., 1916, *66*, 1931.
10 Mendel, L. B., and Benedict, S. R.: Am. J. Physiol., 1909, *25*, 1.
11 Morgan, T. H.: Arch. f. Entwicklungsmech. d. Organ., 1903, *16*, 691.
12 Musselman, L. K.: Proc. Soc. Exper. Biol. & Med., 1929, *27*, 216.
13 Schultze, O.: Arch. f. Entwicklungsmech. d. Organ., 1894, *1*, 269.
14 Spemann, H.: Arch. f. Entwicklungsmech. d. Organ., 1903, *16*, 551.
15 Stockard, C. R.: J. Exper. Zool., 1909, *6*, 285.
16 Stockard, C. R.: Am. J. Obs., 1909, *59*, 582.
17 Wilder, H.: Am. J. Anat., 1908, *8*, 355.