SCIENTIFIC SECTION

THE ABSORPTION OF CALCIUM.*

(A PRELIMINARY REPORT.)

BY A. RICHARD BLISS, JR.,[1] ELLSWORTH O. PRATHER, JR.,[2] AND ROBERT W. MORRISON.[3]

INTRODUCTION.

The metabolism of calcium is intimately related to the calcium and phosphorus content of the ingested food, the activity of the parathyroids, vitamin D and possibly vitamins A and C. Normally, the blood serum contains from 9.0 to 11 mg. of calcium per 100 cc. (1); but in sickness and wasting diseases the amount is lower. In some forms of tetany (2) it has been found occasionally less than half the normal. Updegraff, Greenberg and Clark (3) found that approximately 50 per cent of the total serum calcium is *non-diffusible* or bound calcium, and the remainder is *diffusible;* but Kugelmass and Shohl (4) showed that only a portion of the diffusible calcium is ionized. Some are of the opinion that *ionic calcium* is the active portion, and that the serum calcium is consequently not a true index of the calcium balance. Since ionic calcium diminishes cell permeability (5), excessive amounts decrease cellular activity.

It has been estimated that 99 per cent of the calcium in the body is stored in the bones and teeth as phosphate and carbonate. These compounds provide a reserve to keep the blood calcium normal. Vitamin D is concerned in the development of the teeth, and Bauer and Marble (6) have demonstrated that this vitamin increases the absorption of calcium and phosphorus from the gastro-intestinal tract. In low calcium supply and in chronic states of debility, the bones slowly atrophy through being thus drawn upon for calcium. In acidosis the bone calcium is apparently not readily available. Although the average adult requires about 0.45 Gm. of calcium a day, Sherman (7), to insure the optimum concentration in the blood for both adults and children, advises a minimum of 1 Gm. Sherman and Booher (8) have shown that in growing children the bones may grow in size while calcium poor. During lactation and in the last months of pregnancy, the mother requires double the usual supply of calcium, and, if it is not furnished by the food, loses it from her own bones and teeth. Rickets is associated with inadequate calcification of the bones, and is dependent on derangement of calcium and phosphorus metabolism. It is well known that premature infants are especially predisposed to rickets, since they have less than the normal supply of calcium and phosphorus at birth.

Absorption of calcium takes place in the intestines, but it is influenced by a number of factors. Absorption is *diminished* by: (A) an *excess of phosphorus* in

* Scientific Section, A. PH. A., Madison meeting, 1934.

[1] Director of the Reelfoot Lake Biological Station, Reelfoot Lake, Tennessee.

[2] Instructor in Pharmacology, University of Tennessee.

[3] Associate Professor of Pharmacology, University of South Carolina.

the food, resulting in the formation of the unabsorbable tribasic calcium phosphate; (B) an *excess of fat* in the diet or the incomplete utilization of fat, resulting in the formation of insoluble calcium soaps; (C) *intestinal alkalinity,* as from the ingestion of alkalies, or after meals when there is an abundance of the alkaline pancreatic and intestinal juices and bile (Zucker and Matzner (9) by adding sodium bicarbonate to the diet produced rickets in rats); (D) *hurried loose stools,* as in diarrheas; (E) *stools bulky with cellulose* and roughage. The greater the bulk of the feces, following the ingestion of bran, carrots, spinach, agar, and cellulose flour for bulk, the greater is the loss of calcium.

The absorption of calcium is *favored* by: (A) *intestinal acidity,* as that which may follow the administration of diluted acids, acidified milks and acidotic diets (Aub, *et al.* (10) claim that an acidotic diet may double the urinary calcium, an indication of increased absorption; but if a neutralizing amount of sodium bicarbonate is given with this diet, the urinary calcium is not increased); (B) *the addition of lactose to the food,* which according to Roe and Kahn (11) is probably due to the formation of lactic acid in the intestines.

Calcium chloride and calcium lactate have been extensively studied to determine their absorbability when given orally. Clark (12) with rabbits, Kramer and Howland (13) with rats, and Denis and Minot (14) and a number of others with humans, obtained no significant rise in the blood calcium. However, some observers have reported their absorption. From single doses of 5 Gm. in each of nine human subjects, Aub, Bauer, Heath and Roper (15) reported a rise of 5 to 14 per cent, and from 10 Gm. in eight subjects, a rise of 5 to 28.5 per cent. The maximum rise was found between the first and the fifth hours, and in thirteen of the seventeen subjects, the calcium did not return to the pre-ingestion level for twelve hours. With 5 Gm. doses of the lactate dissolved in water and given on an empty stomach, Roe and Kahn (11) obtained in ten men an average maximum rise in blood calcium of 80 per cent at six to seven hours, the calcium remaining above normal for nine hours; while with 2 Gm. doses they obtained an average maximal rise of 41 per cent at six hours, the calcium remaining above the pre-ingestion level for one and one-half hours longer. Mason (16), Myers and Fine (17), and others reported the lactate inefficiently and the chloride readily absorbed.

According to Meltzer (18), the most effective way to deprive the body of calcium is to give magnesium. Mendel and Benedict (19) and others have demonstrated that magnesium forces calcium from the system. It appears that an excess of magnesium ion exercises a marked specific inhibitory effect on calcification (20, 21, 22, 23). Kramer, *et al.* (24) found that, following the administration of magnesium chloride or sulphate, the inhibition of calcification began when the magnesium of the blood rose above normal. They reported also that as the inorganic phosphorus increased more magnesium was required for the inhibition. It appears that there may be some hazard in the repeated administration of milk of magnesia, at least to children or to those with fractured bones. A diet deficient in calcium is the more harmful if it is at the same time rich in magnesium. Since the magnesium of the cereal grains is mostly in the bran, some pediatricians advise against bran and whole wheat for children. In milk and many vegetables the calcium is high and the magnesium low.

THE METHOD.

The method is based on the antagonism between magnesium and calcium, which, in earlier experiments was observed in animals narcotized by magnesium and awakened by injections of calcium chloride. In these experiments white mice were used. The calcium salts were given by mouth, and, after a certain length of time, the magnesium sulphate ($MgSO_4.7H_2O$) was injected subcutaneously. At the same time controls were run. If some of the ingested calcium were absorbed, then a greater amount of magnesium sulphate is necessary to produce narcosis than is the case where no calcium is given or absorbed.

For from twelve to fifteen hours before the calcium salts were administered the mice received no food or water. The calcium salts were given in 3% to 10% aqueous solutions or suspensions injected into the stomach by means of a thin stomach tube fitted with a small syringe. The time interval between the feeding of the calcium and the injection of the magnesium was two hours. Magnesium sulphate was injected subcutaneously in a 10% aqueous solution. A definite degree of narcosis was required which the authors determined to be at the point where the mice lie on their backs without movement or attempts to turn over. This degree of narcosis is usually reached by effective doses in from twelve to twenty minutes after the injection.

On the basis of an extensive series of preliminary experiments (Table I), 0.9 mg. of magnesium sulphate per Gm. of body weight proved to be just sufficient to produce the desired state of narcosis in the mice. The amount of each calcium salt administered in mg. per Gm. of body weight is equivalent to 0.3 mg. of calcium per Gm. of body weight.

TABLE I.—MAGNESIUM SULPHATE ($MgSO_4.7H_2O$).

Wt. (Gm.) of Mouse.	Dose of $MgSO_4$ Mg. per Gm.	Effect.	Time to Action.
18.27	0.75	None	......
18.04	0.75	None	......
23.81	0.75	None	......
20.32	0.75	None	......
14.75	0.80	Partial narcosis	18 min.
21.30	0.80	"	17 min.
18.70	0.80	"	20 min.
15.25	0.80	"	30 min.
18.70	0.90	"	20 min.
16.45	0.90	"	20 min.
14.62	0.90	"	20 min.
18.24	0.90	"	20 min.
14.70	0.95	"	18 min.
26.56	0.95	Complete narcosis	16 min.
17.20	0.95	"	17 min.
18.00	0.95	"	16 min.
14.00	1.00	Complete narcosis	20 min.
15.70	1.00	"	18 min.
25.65	1.00	"	17 min.
18.58	1.00	"	17 min.
18.63	1.50	"	14 min.
20.46	1.50	"	12 min.
17.00	1.50	"	7 min.
18.40	1.50	"	10 min.

TABLE II.—CALCIUM CHLORIDE.

Wt. (Gm.) of Mouse.	Mg. of $CaCl_2$, per Gm. Body Wt.	Dose (Mg. per Gm.) of $MgSO_4$ 2 Hrs. after Ca.	Time to Narcosis.
20.85	0.82425	1.0	......
21.75	"	1.0	......
21.84	"	1.0	......
16.77	"	1.25	Partial; 20 min.
23.55	"	1.25	Partial; 31 min.
28.50	"	1.25	Partial; 26 min.
23.75	"	1.50	Complete; 18 min.
19.52	"	1.50	Complete; 15 min.
18.60	"	1.50	Complete; 13 min.

TABLE III.—CALCIUM LACTATE.

Wt. (Gm.) of Mouse.	Mg. of Lact., per Gm. Body Wt.	Dose (Mg. per Gm.) of $MgSO_4$ 2 Hrs. after Ca.	Time to Narcosis.
24.37	1.63635	0.90	......
19.61	"	0.90	......
22.98	"	0.90	......
24.76	"	1.00	......
21.86	"	1.00	......
22.86	"	1.00	Partial
23.70	"	1.25	"
22.40	"	1.25	"
17.38	"	1.25	"
18.77	"	1.50	Partial
20.41	"	1.50	Complete; 20 min.
22.43	"	1.50	Complete; 17 min.
21.57	"	2.00	Complete; 10 min.
17.82	"	2.00	Complete; 11 min.
22.50	"	2.00	Complete; 15 min.

TABLE IV.—CALCIUM GLUCONATE.

Wt. (Gm.) of Mouse.	Mg. of Glucon., per Gm. Body Wt.	Dose (Mg. per Gm.) of $MgSO_4$ 2 Hrs. after Ca.	Time to Narcosis.
18.91	3.364215	0.90	......
21.43	"	0.90	......
17.19	"	0.90	......
18.23	"	1.00	Partial; 20 min.
18.23	"	1.00	Partial; 30 min.
22.83	"	1.00	Partial; 24 min.
22.00	"	1.25	Partial; 20 min.
21.00	"	1.25	Partial; 22 min.
22.72	"	1.25	Partial; 20 min.
21.67	"	1.50	Partial; 20 min.
17.00	"	1.50	Complete; 20 min.
18.62	"	1.50	Complete; 20 min.
23.16	"	2.00	Complete; 14 min.
16.95	"	2.00	Complete; 11 min.
18.82	"	2.00	Complete; 12 min.

TABLE V.—CALCIUM DIPHOSPHATE.

Wt. (Gm.) of Mouse.	Mg. of Diphos. per Gm. Body Wt.	Dose (Mg. per Gm.) of $MgSO_4$ 2 Hrs. after Ca.	Time to Narcosis.
16.82	1.29375	0.9	......
21.69	"	0.9	......
17.52	"	0.9	......
19.66	"	1.0	Partial
15.15	"	1.0	"
21.57	"	1.0	"
21.00	"	1.25	Partial; 20 min.
17.30	"	1.25	Complete; 13 min.
21.30	"	1.25	Complete; 12 min.
17.67	"	1.50	Complete; 12 min.
23.77	"	1.50	Complete; 10 min.
20.46	"	1.50	Complete; 5 min.

TABLE VI.—CALCIUM GLYCEROPHOSPHATE.

Wt. (Gm.) of Mouse.	Mg. of Glyceroph. per Gm. of Body Wt.	Dose (Mg. per Gm.) of $MgSO_4$ 2 Hrs. after Ca	Time to Narcosis.
20.34	1.6115	0.9	Partial
19.50	"	0.9	"
16.85	"	0.9	"
15.12	"	1.0	Complete; 20 min.
16.10	"	1.0	Partial
18.00	"	1.0	Complete; 8 min.
19.00	"	1.25	Complete; 20 min.
19.50	"	1.25	Complete; 9 min.
22.72	"	1.25	Complete; 8 min.
23.00	"	1.50	Complete; 7 min.
20.09	"	1.50	Complete; 11 min.
16.54	"	1.50	Complete; 12 min.

TABLE VII.—INOSITE HEXACALCIUM GLUCONATE.

Wt. (Gm.) of Mouse.	Mg. of Inosite Comp. per Gm. Body Wt.	Dose (Mg. per Gm.) of $MgSO_4$ 2 Hrs. after Ca.	Time to Narcosis.
16.82	1.20096	0.9	......
21.82	"	0.9	......
19.77	"	0.9	......
17.62	"	1.0	......
18.00	"	1.0	Partial; 20 min.
21.76	"	1.0	Partial; 20 min.
17.18	"	1.0	Partial; 20 min.
18.75	"	1.0	Partial; 20 min.
17.56	"	1.0	Partial; 20 min.
15.00	"	1.25	Partial; 20 min.
16.37	"	1.25	Partial; 20 min.
15.00	"	1.25	Complete; 20 min.
18.66	"	1.25	Partial; 20 min.
18.80	"	1.25	Partial; 20 min.
20.17	"	1.25	Complete; 15 min.
20.00	"	1.25	Complete; 13 min.
18.00	"	1.50	Complete; 10 min.
20.00	"	1.50	Complete; 20 min.
22.86	"	1.50	Complete; 20 min.

21.80	"	1.50	Complete; 19 min.
22.85	"	1.50	Complete; 13 min.
17.44	"	1.50	Complete; 11 min.

TABLE VIII.—SUMMARY.

Drug.	Amt. $MgSO_4$ Causing Partial Narcosis.	Amt. $MgSO_4$ Causing Complete Narcosis.
Calcium chloride	1.25* (3 out of 3)	1.50* (3 out of 3)
Calcium lactate	1.00 (1 out of 3)	
	1.25 (3 out of 3)	
	1.50 (1 out of 3)	1.50 (2 out of 3)
		2.00 (3 out of 3)
Calcium gluconate	1.00 (3 out of 3)	
	1.25 (3 out of 3)	
	1.50 (1 out of 3)	1.50 (2 out of 3)
		2.00 (3 out of 3)
Calcium diphosphate	1.00 (3 out of 3)	
	1.25 (1 out of 3)	1.25 (2 out of 3)
		1.50 (3 out of 3)
Calcium glycerophosphate	0.90 (2 out of 3)	
	1.00 (1 out of 3)	1.00 (2 out of 3)
		1.25 (3 out of 3)
		1.50 (3 out of 3)
Inosite hexacalcium gluconate	1.00 (5 out of 5)	
	1.25 (4 out of 7)	1.25 (3 out of 7)
		1.50 (6 out of 6)

* mg. $MgSO_4.7H_2O$ per Gm. of mouse.

DISCUSSION.

In Table I are shown the effects produced by various amounts of magnesium sulphate ($MgSO_4.7H_2O$). Nine-tenths of a milligram (0.9 mg.) per Gm. of body weight was shown to be the amount required to produce the desired state of narcosis in white mice.

Tables II through VIII show the effects produced by magnesium sulphate in various doses following the oral administration of equivalent quantities (Ca) of the six calcium compounds studied. By subtracting 0.9 mg. from the magnesium sulphate values given in these tables (II–VIII), the amounts of magnesium sulphate required to completely antagonize the *absorbed* calcium are obtained. The greater the quantity of magnesium sulphate required to produce the narcosis, the larger the amount of *absorbed* calcium.

When arranged according to relative efficacy the calcium compounds used in this study place themselves in the following order:

(1) Calcium lactate
(2) Calcium gluconate
(3) Calcium chloride
(4) Inosite hexacalcium gluconate
(5) Calcium diphosphate
(6) Calcium glycerophosphate.

When arranged quantitatively according to the actual amounts of the calcium compounds themselves administered and expressed in grams per Gm. of body weight of mouse, the order is:

(1)	Calcium chloride	0.00082425
(2)	Inosite hexacalcium gluconate	0.00120096
(3)	Calcium diphosphate	0.00129375
(4)	Calcium glycerophosphate	0.00161150
(5)	Calcium lactate	0.00163635
(6)	Calcium gluconate	0.00336241

The foregoing quantities represent equivalent amounts of calcium.

Attention is called to the fact that this preliminary study leaves relatively wide gaps between the various quantities of magnesium sulphate used to neutralize the absorbed calcium (gaps between 1.0 and 1.25, 1.25 and 1.50, 1.50 and 2.00); and also that but one time interval (2 hours) was involved. Consequently further studies are underway in which the effects of longer time intervals between the doses of the calcium compounds and the doses of the magnesium sulphate are being recorded, as well as the effects of increases of 0.1 mg. of magnesium sulphate per Gm. of body weight of mouse between the values (1.0 and 1.25, 1.25 and 1.50, 1.50 and 2.00) which appear in the present tables.

BIBLIOGRAPHY.

(1) A. Cantarow, "Calcium Metabolism and Calcium Therapy," 2nd Edition (1933), 39.
(2) A. Cantarow, *Ibid.*, 2nd Edition (1933), 136.
(3) H. Updegraff, D. M. Greenberg and G. W. Clark, *J. Biol. Chem.*, 71 (1926), 87.
(4) I. Kugelmass and A. T. Shohl, *Ibid.*, 58 (1923–1924), 649.
(5) J. Loeb, *Am. J. Physiol.*, 5 (1901), 362.
(6) W. Bauer and A. Marble, *J. Exptl. Med.*, 49 (1929), 145.
(7) H. C. Sherman, "Chemistry of Foods and Nutrition," 4th Edition (1932).
(8) H. C. Sherman and L. E. Booher, *J. Biol. Chem.*, 93 (1931), 93.
(9) T. F. Zucker and M. J. Matzner, *Proc. Soc. Exptl. Biol. Med.*, 21 (1923–1924), 186.
(10) J. C. Aub, W. Bauer, C. Heath and M. Roper, *J. Clin. Investigation*, 7 (1929), 97.
(11) J. H. Roe and B. S. Kahn, *J. A. M. A.*, 88 (1927), 981.
(12) G. W. Clark, *J. Biol. Chem.*, 43 (1920), 89.
(13) B. Kramer and J. Howland, *Ibid.*, 43 (1920), 35.
(14) W. Denis and A. S. Minot, *Ibid.*, 41 (1920), 357.
(15) J. C. Aub, W. Bauer, W. Heath and M. Roper, *J. Clin. Investigation*, 7 (1929), 97.
(16) E. H. Mason, *J. Biol. Chem.*, 67 (1926), 71.
(17) V. C. Myers and M. S. Fine, *Proc. Soc. Exptl. Biol. Med.*, 16 (1919), 73.
(18) S. J. Meltzer, *Ibid.*, 10 (1913), 159; and *J. Pharm.*, 12 (1918), 211.
(19) L. B. Mendel and S. R. Benedict, *Am. J. Physiol.*, 25 (1909–1910), 23.
(20) J. Novi, *Zentr. Biochem. Biophys.*, 13 (1912), 578.
(21) J. Hellwig, *Z. Biol.*, 73 (1921), 281.
(22) E. B. Hart and K. Steenboch, *J. Biol. Chem.*, 14 (1925), 75.
(23) I. Greenwald and J. Gross, *Ibid.*, 66 (1925), 185, and 201; 68 (1926), 325.
(24) B. Kramer, F. F. Tisdall and J. Howland, *Am. Jour. Diseases Children*, 22 (1921), 560.

THE SOLUBILITY OF CALCIUM LEVULINATE IN WATER.

BY GERALD J. COX,[1] MARY L. DODDS[2] AND CLARENCE CLASPER.[3]

Calcium levulinate has been shown to be more satisfactory for parenteral administration of calcium than any other known salt (1, 2). Anticipating the prob-

[1] Senior Industrial Fellow. [2] Industrial Fellow. [3] Fellowship Assistant.