マグネシウムの沈澱形成に及ぼすpHおよびカルシウムとリン含量の影響

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EFFECTS OF pH, Ca AND P ON THE FORMATION OF Mg PRECIPITATE

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I. INTRODUCTION

It has been suggested that hypomagnesemia in ruminants may be induced by the failure of magnesium (Mg) absorption in the gastrointestinal tract. The decrease of Mg absorption is considered by the precipitate formations of Mg with ammonia (8, 9), organic acids (3, 10, 18), and phosphorus (P) (12), and by the competition with potassium (K) (4, 11, 12) or calcium (Ca) (2, 11, 18) for the absorption site in the gastrointestinal tract. In the previous papers (15-17, 19, 20), the positive correlations were found among Mg, Ca and P contents in the feces of ewes (15, 19, 20) and in digesta of rats (16, 17), and the precipitate formation of those minerals was remarkably influenced by pH of digesta (17). On the other hand, phosphoric acid ions (HPO_4^{2-} and PO_4^{3-}) combine easily with Mg or Ca ions in alkalinity to form insoluble phosphates (7). This suggests that the absorption of Mg and Ca may be influenced by P content and pH of digesta in the gastrointestinal tract. However, the interrelationships among pH, Mg, Ca and P in digesta are not fully understood yet.

In the present study, the effects of pH, Ca and P on the formation of Mg precipitate were examined as a preliminary step to clarify the Mg availability in the gastrointestinal tract of ruminants.

II. MATERIALS AND METHODS

Solution: 27 phosphate buffer solutions (A solution) prepared by the combination of 9 different pH values and 3 different concentrations of P were adjusted with 0.20 M disodium monohydrogenphosphate ($Na_2HPO_4 \cdot 12H_2O$; Kanto Chemical Co. Inc.) and 0.20 M potassium dihydrogenphosphate (KH_2PO_4; Kanto Chemical Co. Inc.) (Table 1). In addition, 5 solutions of different Mg/Ca ratios (B solution) were adjusted with 0.10 M magnesium chloride ($MgCl_2 \cdot 6H_2O$; Kanto Chemical Co. Inc.) and 0.10 M calcium chloride ($CaCl_2 \cdot 2H_2O$; Kanto Chemical Co. Inc.).

Procedures: 135 various solutions (total volume of 4.0 m*l*) which were prepared by mixture of equal quantity of A and B solutions were reacted at 37°C for 1 hr

Table 1. Experimental design of the solutions

pH		P content (mol/4.0m*l*)		Mg and Ca contents (mol/4.0m*l*)		
				Mg	Ca	Mg+Ca
① 4.40				(a) 0.0500	0	0.0500
② 6.00						
③ 6.50		(I) 0.0250		(b) 0.0375	0.0125	0.0500
④ 6.75						
⑤ 7.00	×	(II) 0.0500	×	(c) 0.0250	0.0250	0.0500
⑥ 7.25						
⑦ 7.50		(III) 0.1000		(d) 0.0125	0.0375	0.0500
⑧ 8.00						
⑨ 9.00				(e) 0	0.0500	0.0500

Note: Solutions (135 kinds) were prepared by the combination of 9 different pHs (①-⑨), 3 different contents of P (I-III) and 5 different Mg/Ca ratios (a-e).

(Table 1), and was centrifuged at 3,000 rpm for 10 min. The supernatant was immediately used for analysis. These steps were repeated 4 times.

Analysis: Mg and Ca contents were analysed with an atomic absorption spectrophotometer (Type 508, Hitachi, Ltd.). P content was measured by the colorimetric method of GOLDENBERG (1).

The precipitate (ppt) ratios of Mg, Ca and P were calculated by the following equation: (total−supernatant contents)×100/total content (%). The values obtained were statistically treated with Student's *t* test and applied to multiple regression analysis.

III. RESULTS

Mg: In the solutions containing Mg alone (a-I～III), the ppt of Mg was not formed in acidity or neutrality, except for the a-III solution at pH 7.00 in which a slight formation of Mg precipitate was observed (Fig. 1). In alkalinity, however, the ppt of Mg was formed according to the increase of pH and P content. The ppt ratio of Mg reached a plateau at pH 9.00. These plateau levels were significantly different from each other ($P<0.01$).

In the solutions containing both Mg and Ca (b-I～III, c-I～III and d-I～III), the formation of the ppt of Mg bigan at pH 6.50. The ppt ratio of Mg increased with increase of pH and P content and it reached a maximum level at pH 9.00 (Fig. 1). In the a～d-I and -II solutions, the ppt ratio of Mg at pH 9.00 declined with the decrease of Mg/Ca ratio. In the a～d-III solutions, however, the ppt ratio of Mg at pH 9.00 increased with decrease of Mg/Ca ratio. The maximum level in the a-III solution was significantly higher than that in the b- and c-III solutions ($P<0.01$).

Ca: In the solutions of e-I～III which contained Ca alone, the ppt of Ca began

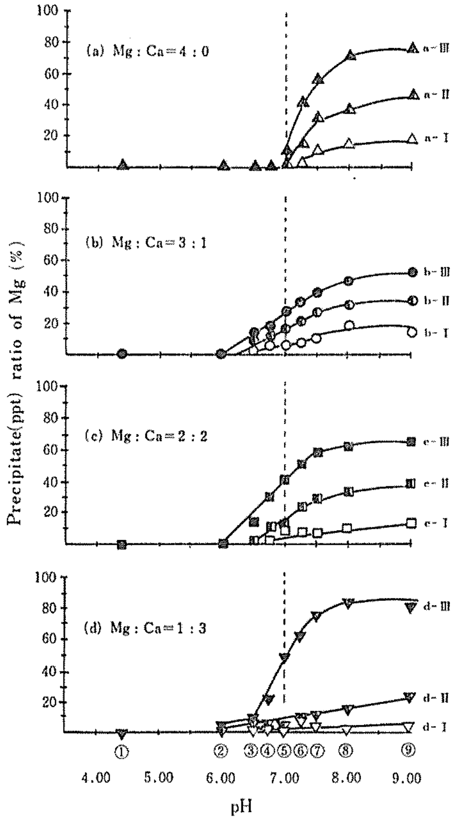

Fig. 1. Effects of pH, Ca and P in the solution on the precipitate (ppt) ratio of Mg.

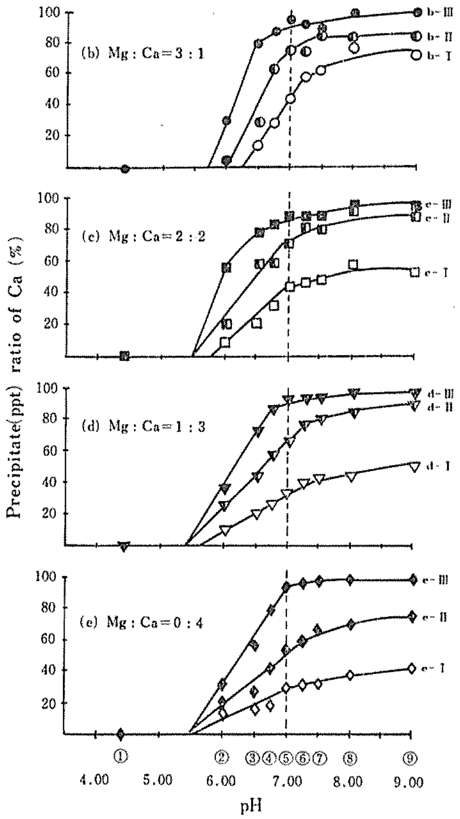

Fig. 2. Effects of pH, Mg and P in the solution on the precipitate (ppt) ratio of Ca.

at pH 6.00. The ppt ratio of Ca increased with the increase of pH and P content, reaching a maximum level at pH 9.00 (Fig. 2). The maximum level at pH 9.00 in the e-I～III solutions differed significantly between each other ($P<0.01$, respectively).

In the b-, c- and d-I～III solutions containing both Mg and Ca, the formation of the ppt of Ca began at pH 6.00. The ppt of Ca increased with the increase of pH and P content, reaching a maximum level at pH 7.50 or 9.00 in the b-, c- and d-I～III solutions, as in the e-I～III solutions (Fig. 2). In the solutions of low P contents (b～e-I), the maximum levels of the ppt ratio of Ca decreased significantly as the Mg/Ca ratio decreased. In the solutions of middle and high P contents (b～e-II and -III), however, the maximum levels of the ppt ratio of Ca were remarkably high in the II and III solutions (85.8～100.0%), except for the e-II solution (75.2%).

P: The ppt ratios of P changed depending on the contents of Mg and/or Ca in all solutions (a-I～III to e-I～III) (Fig. 3).

Relationships among Mg, Ca and P: Significantly positive correlations were noted between the ppt ratios of P and Mg or Ca in all solutions except d-I (Table 2), and between the ppt contents of Mg and Ca in parts of the solutions (Table 3).

Furthermore, there were significantly positive correlations observed between pH and the ppt ratio of P to Mg plus Ca in a～e-II and -III solutions from pH 6.00 to 9.00, but there was no significant correlation between pH and the ppt ratio of them in a～e-I solution (Fig. 4).

Table 2. Correlations between the precipitate (ppt) ratios of Mg or Ca and P in the solutions

Solution[1)]	Regression equation		Regression equation	
(n=9)[2)]	Mg(X) vs. P(Y)	r[3)]	Ca(X) vs. P(Y)	r
a-I	y=2.03x− 3.23	0.9860***		
a-II	y=0.85x+ 0.23	0.9967***		
a-III	y=0.43x+ 0.03	0.9994***		
b-I	y=2.88x+ 5.87	0.9093***	y=0.66x− 1.10	0.9932***
b-II	y=1.22x+ 3.94	0.9564***	y=0.45x− 0.37	0.9751***
b-III	y=0.47x+ 6.32	0.9203***	y=0.26x− 0.92	0.9686***
c-I	y=4.35x+13.66	0.8869**	y=1.04x− 1.04	0.9709***
c-II	y=1.06x+17.37	0.8633**	y=0.57x+ 0.03	0.9935***
c-III	y=0.37x+11.57	0.8725**	y=0.36x− 1.43	0.9768***
d-I		NS[4)]	y=1.31x+ 0.59	0.9990***
d-II	y=2.34x+18.39	0.7773*	y=0.67x− 1.83	0.9936***
d-III	y=0.38x+10.35	0.9250***	y=0.40x− 0.06	0.9900***
e-I			y=1.66x− 1.72	0.9784***
e-II			y=0.84x− 0.75	0.9930***
e-III			y=0.41x+ 1.90	0.9927***

Note: 1) See Table 1 2) Sample numbers (from pH 4.40 to 9.00) 3) Correlation coefficient 4) Not-significant *, **, *** P<0.05, 0.01 and 0.001, respectively

Table 3. Correlations between the precipitate (ppt) ratios of Mg and Ca in the solutions

Solution[1)]	Range of pH	(n)[2)]	Regression equation Mg(X) vs. Ca(Y)	r[3)]
b-I	4.40-6.75	(4)	y=6.52x− 1.01	0.9967**
	7.25-9.00	(4)	y=1.68x+46.67	0.9885*
b-II	4.40-6.75	(4)	y=4.97x− 3.30	0.9873*
b-III	4.40-6.75	(4)	y=4.52x+10.12	0.9719*
d-II	7.25-9.00	(4)	y=0.88x+69.78	0.9524*

Note: 1) See Table 1 2) Sample numbers 3) Correlation coefficient *, ** P<0.05 and 0.01, respectively

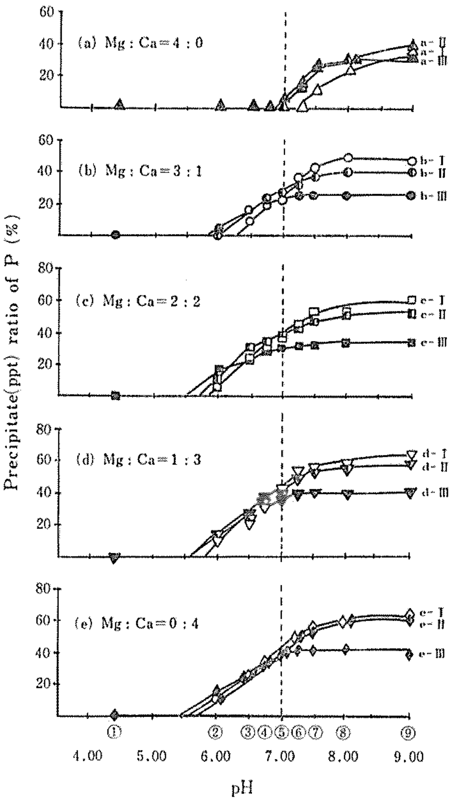

Fig. 3. Effects of pH, Mg and Ca in the solution on the precipitate (ppt) ratio of P.

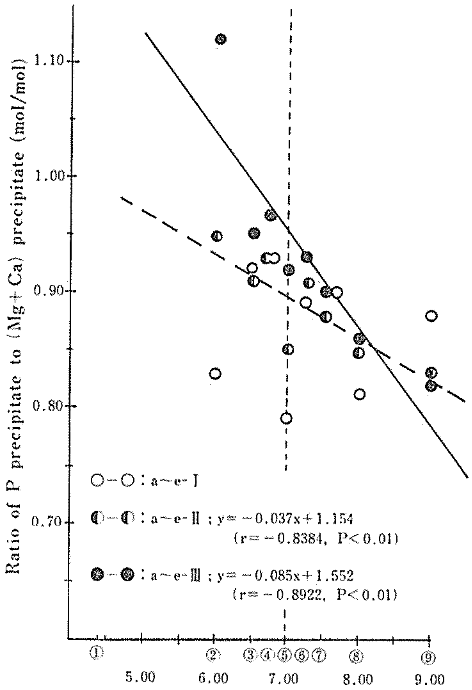

Fig. 4. Correlation between pH and the precipitate (ppt) ratio of P to Mg plus Ca in the solution.

VI. DISCUSSION

In the present experiment, Mg was not precipitated in acidity, but did suddenly over pH 7.00 in the solutions containing Mg alone (Fig. 1). The ppt of Mg increased with the increase of pH and P content, reaching a maximum level at pH 8.00 or 9.00. Since Mg ions (Mg^{2+}) react with phosphate ions (HPO_4^{2-} or PO_4^{3-}) to form insoluble magnesium phosphates [$MgHPO_4$ or $Mg_3(PO_4)_2$] (7), the ppt formation of Mg with P might be remarkably influenced by pH and P content.

The 3 solutions (b, c and d) of different Mg/Ca ratios were mixed with the phosphate solutions to examine ppt formation of Mg. The Mg ppt was formed from pH6.50, being different from the solutions of Mg alone. The Mg ppt ratio in each solution increased continuously with the increase of pH and P content, and it reached a maximum level at pH 8.00 or 9.00, as in the solutions of Mg alone. The ppt of Mg formed in acidity might be composed of insoluble complexes containing Ca and P, because it was not formed in the solutions containing Mg and phosphates in acidity and there were significantly positive correlations among the ppt ratios of Mg, Ca and P between pH 4.40 and 7.00 (Figs. 1-3 and Table 2). In alkalinity, the ppt ratio of Mg in I and II solutions decreased with the decrease of P content and Mg/Ca ratios (Fig. 1). This result showed that the binding force of Mg with P was much weaker than that of Ca with P because of competition between Mg^{2+} and Ca^{2+} for phosphate ions (HPO_4^{2-} or PO_4^{3-}). The ppt ratio of Mg increased with the decrease of Mg/Ca ratio in the P content which was more than Mg plus Ca content (III-b$<$-c$<$-d), because the ppt of Mg with P more than Mg plus Ca content appeared to be formed without the competition by Ca ions (Figs. 1 and 2). However, it was demonstrated that the ppt formation of Mg was remarkably influenced by Ca/P ratio and pH (especially in weak alkalinity), because the maximum ppt ratio of Mg increased with the decrease of Mg/Ca ratio (b$<$c$<$d) in spite of high ppt ratio of Mg in the solution containing Mg alone (about 80 %). In the previous paper (17), the ppt ratio of Mg in digesta in the gastrointestinal tract of rats increased with the increase of pH. It was reported that Mg solubility was remarkably influenced by pH, Ca and P in solutions (21). Those results indicated that the availability of Mg in animals might be affected also by pH, Ca and P of digesta in the gastrointestinal tract.

On the other hand, Ca was precipitated at pH 6.00, and the ppt of Ca gradually increased with the increase of pH and P content, reaching a maximum level at pH 8.00 or 9.00 (Fig. 2). This result indicated that Ca began to react with phosphates in weak acidity to form insoluble calcium phosphates [$CaHPO_4$ or $Ca_3(PO_4)_2$] (7).

In the solutions (b, c and d) of different Mg/Ca ratios, the ppt ratio of Ca increased with the decrease of Mg/Ca ratio in the P content which was less than or equal to Mg plus Ca content (I and II) (Fig. 2). And it reached the plateau level of about 100 % in the solution of which P content was more than the Mg plus Ca content regardless of Mg/Ca ratio (III). Thus, it was clear that the ppt formation of Ca was hardly influenced by the presence of Mg, but was influenced directly by P content and pH (especially in acidity). Difference between the ppt ratios of Mg and Ca might be due to the difference of the ionization tendency between Mg and Ca (Figs. 1 and 2).

There were significantly positive correlations found between the ppt ratios of P and Mg or Ca in all solutions (Table 4). This suggested that the ppt of P was formed with Mg and Ca. However, P/Mg+Ca ratio changed depending on pH (0.81-1.12) (Fig. 4). Since it is known that the forms of phosphate ions are changed by pH ($H_2PO_4^- \rightleftharpoons HPO_4^{2-} \rightleftharpoons PO_4^{3-}$) (7), it was suggested that the ratio of PO_4^{3-} to HPO_4^{2-} might increase with the increase of pH ($HPO_4^{2-} : Mg^{2+}+Ca^{2+}=1:1=1.00 \rightarrow PO_4^{3-} : Mg^{2+}+Ca^{2+}=2:3=0.67$), and consequently, the ppt contents of Mg and Ca might increase in alkalinity.

The grazing ruminants which are fed orchard grass containing low Mg and Ca content in the early spring (6, 13, 20) may be easily susceptible to hypomagnesemia, because ruminants secrete large amount of alkali saliva containing high P content and the digesta in the gastrointestinal tract of it were largely in alkalinity (14).

V. SUMMARY

One handred and thirty five various solutions prepared by the combination of 9 different pHs, 3 different P contents and 5 different Mg/Ca ratios were reacted at 37°C for 1 hr to determine the effects of pH, Ca and P on the formation of Mg precipitate (ppt). In the solutions containing Mg alone, Mg was not precipiated in acidity but in alkalinity. The ppt ratio of Mg increased with the incease of pH and P content. In the solutions of different Mg/Ca ratios, the ppt of Mg was formed in acidity and the ppt ratio of Mg increased with the increase of pH and P content in alkalinity. In Mg+Ca≧P contents, the higher the Mg/Ca ratio was, the lower the ppt ratio of Mg was. In Mg+Ca<P contents, however, the ppt ratio of Mg was high. In the solutions containing Ca alone, the ppt of Ca was formed in acidity and the ppt ratio of Ca increased with the increase of pH and P content in acidity. In the solutions of different Mg/Ca ratios, the ppt ratio of Ca increase of pH and P content, and was not influenced by the presence of Mg. Significantly positive correlations were observed between the ppt ratio of P and Mg or Ca. These results suggested that the ppt formaion of Mg in the gastrointestinal tract of ruminants might be remarkably influenced by pH, Ca and P of digesta.

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REFERENCES

1. BABA, S. and K. OKUDA (1973) Methods in Medical Chemistry Vol. 3B. Nakayama-shoten, Tokyo : 359-362 (Japanese).
2. BEHAR, J. (1975) Effect of calcium on magnesium absorption. Am. J. Physiol. **229** : 1590-1595.
3. BURT, A. W. and D. C. THOMAS (1961) Dietary citrate and hypomagnesaemia in the ruminant. Nature **192** : 1193.
4. CARE, A. D. and J. DUNCAN (1967) Factors affecting magnesium absorption in relation to the aetiology of acute hypomagnesaemia. J. Agr. Sci. **68** : 195-204.
5. CHICCO, C. F., C. B. AMMERMAN, J, P. FEASTER and B. G. DUNAVANT (1973) Nutritional interrelationships of dietary calcium, phosphorus and magnesium in sheep. J. Anim. Sci. **36** : 986-993.
6. GRUNES, D. L., P. R. STOUT and J. R. BROWNELL (1970) Grass tetany of ruminants. Advances in Agronomy **22** : 331-374.

7. HASHIMOTO, T. (1981) Sanseidojyo to sakumotsuseiiku [Acid Earth and Growth of Crops]. Bun-eido, Tokyo: 36–38 (Japanese).

8. HEAD, M. J. and J. A. F. ROOK (1955) Hypomagnesemia in dairy cattle and its possible relationships to ruminal ammonia production. Nature **176**: 262–263.

9. HENRY, P. R., W. H. SMITH and M. D. CUNNINGHAM (1977) Effect of histamine and ammonia on hypomagnesaemia in ruminants. J. Anim. Sci. **44**: 276–281.

10. HOUSE, W. A. and D. VAN CAMPEN (1971) Magnesium metabolism of sheep fed different levels of potassium and citric acid. J. Nutr. **101**: 1483–1492.

11. KEMP, A. (1960) Hypomagnesaemia in milking cows. The response of serum Mg to alterations in herbage composition resulting from potash and nitrogen dressing on pasture. Netherl. J. Agr. Sci. **8**: 281–304.

12. PACKETT, L. V. and J. HAUSECHILD (1963) Relationship of magnesium, calcium and phosphorus to incidence of urinary calculi in lambs. J. Anim. Sci. **22**: 843.

13. ROOK, J. A. F. and J. E. STORRY (1962) Magnesium in the nutrition of farm animals. Nutr. Abst. Rev. **32**: 1055–1077.

14. SASAKI, Y. (1966) Daeki to daeki bunpitsu [Saliva and Its Secretion]. *In*: Nyugyu no kagaku [Science of Dairy Cow] (UMEZU, M. ed.). Nosan-gyoson-bunka-kyokai, Tokyo: 79–89 (Japanese).

15. SHIGA, A., A. KOMINATO and K. SHINOZAKI (1980) Experimental studies on hypomagnesemia in ruminants. V. Metabolism of phosphorus and plasma level of parathyroid hormone in sheep fed diets of varying composition of magnesium and calcium. Jpn. J. Vet. Sci. **42**: 221–230 (Japanese with English summary).

16. SHIGA, A. and Y. MORINO (1986) Correlations among Mg, Ca and P contents of digesta in the gastro-intestinal tract of rats. Jpn. J. Vet. Sci. **48**: 1283–1286.

17. SHIGA, A., T. SASAKI and N. HORII (1987) Correlations among pH and Mg, Ca, P, Na, K, Cl^- and HCO_3^- contents of digesta in the gastro-intestinal tract in rats. Jpn. J. Vet. Sci. **49**: 973–979.

18. SHIGA, A. and K. SHINOZAKI (1975) Studies on calcium and magnesium metabolism in ruminants. I. Effects of calcium carbonate or sodium oxalate on the absorption and excretion of calcium and magnesium in ewes. J. Fac. Agr. Iwate Univ. **12**: 379–389 (Japanese with English summary).

19. SHIGA, A. and K. SHINOZAKI (1980) Experimental studies on hypomagnesemia in ruminants. VI. Comparison of metabolism of magnesium, calcium and phosphorus and plasma level of parathyroid hormone in lactating and non-lactating ewes at the time of change from a control diet to a diet low in magnesium and calcium. Jpn. J. Vet. Sci. **42**: 231–241 (Japanese with English summary).

20. SHINOZAKI, K., K. FUJITA and A. SHIGA (1978) Experimental studies on hypomagnesemia of ruminants. I. Mineral balance in sheep at the time of change from winter ration to spring herbage. Jpn. J. Vet. Sci. **40**: 407–414 (Japanese with English summary).

21. SUGAWARA, H. (1977) Effects of several factors on the solubility and the absorbability of magnesium. J. Fac. Agr. Iwate Univ. **13**: 269–287.

マグネシウムの沈澱形成に及ぼす pH および カルシウムとリン含量の影響

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摘 要

反芻動物の低マグネシウム (Mg) 血症に関する研究の一環として，消化管内容中 Mg の物理・化学的性状に及ぼす pH およびカルシウム (Ca) とリン (P) 含量の影響を調べるため，9段階の pH (4.0−9.0)，3段階のP含量 (0.025，0.050，0.100M)，5種類の Mg と Ca の混合比 (Mg+Ca=0.05M，Mg：Ca=4：0，3：1，2：2，1：3 および 0：4) の組み合せによる135種類の溶液 (各4.0ml) を，37°C，1時間反応させ，Mg，Ca，P の沈澱率 [(総量−上清量)÷総量×100(%)] およびそれらの相互関係を求めた．

その結果，以下の成績を得た．

1) Mg は，単独の場合，酸性では沈澱しなかったが，アルカリ性では沈澱し，その沈澱率は pH が高いほど，P含量が多いほど高く，pH 9.0 でほぼ平衡に達した．Ca を含む場合，Mg は弱酸性 (pH 6.5) で沈澱し，その沈澱率は pH が高いほど，P含量が多いほど高かった．Mg の沈澱率は，Mg+Ca<P では Ca の割合が高いほど高かった．

2) Ca は，単独の場合，pH 6.0 で沈澱し，その沈澱率は中性付近で平衡に達した．Mg を含む場合，Ca の沈澱率は，pH が高いほど，P含量が多いほど高かったが，Mg の影響はほとんどなかった．

3) Pの沈澱率は，Mg と Ca の沈澱率との間にそれぞれ有意の正の相関を示した．また，pH の上昇に伴い，沈澱中 HPO_4^{2-} に対する PO_4^{3-} の割合が高まることが示唆された．

以上のことから，消化管内容の Mg の沈澱形成 (利用性) には，pH と Ca およびP含量が密接に関与することが示唆された．