Correlations among pH and Mg, Ca, P, Na, K, Cl^- and HCO_3^- Contents of Digesta in the Gastro-Intestinal Tract of Rats

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ABSTRACT. pH and Mg, Ca, P, Na, K, Cl^- and HCO_3^- contents of digesta in the stomach, the small intestine, the caecum, the colon were determined in the rats to study the effects of pH on absorption of Mg, Ca and P in the gastro-intestinal tract. Acidification in the stomach (pH3.46), sudden pH rise in the upper segment of the small intestine (pH6.52), sudden alkalization from pH6.89 to pH7.41 in the defined site of the lower segment of the small intestine, acidification in the caecum (pH6.91) and sudden alkalization in the colon (pH7.97) were observed. The positive correlations among Cl^- , HCO_3^- , Na and K contents suggested that these minerals neutralized the low pH by HCl secreted in the stomach and organic acids produced by fermentation in the caecum. The significant negative correlations between pH and the ratio of Mg, Ca or P contents (Sup %) in supernatant fraction to total contents showed the possibility that the insoluble salts of these minerals precipitated as pH rose. The sudden increase of P content in the caecum might be due to the accumulation of P into multiplied bacteria. These results suggested that the sudden alkalization of digesta might decrease the absorptions of Mg, Ca and P in the intestine.—**KEY WORDS:** Ca, Mg, P, rat.

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In a previous report [16], the contents of Mg and Ca of digesta were remarkably low in the caecum and in the upper small intestine, respectively, in the rat and positive correlations were observed among Mg, Ca and P contents in various portions of the gastro-intestinal tract.

On the other hand, it is known that phosphoric acid ions (HPO_4^{2-} and PO_4^{3-}) combine easily with Mg and Ca ions in alkalinity to form insoluble salts [10]. Therefore, P content and pH of digesta in the gastro-intestinal tract may affect the absorption of Mg and Ca, which are absorbed only in the ion status. The pH of digesta in the gastro-intestinal tract is mainly regulated by HCl secreted from the parietal cells in the stomach, and alkali salts (NaHCO_3 , KHCO_3 , etc.) from saliva, pancreatic juice, bile and intestinal juice [13]. However, the relationships among pH and Mg, Ca and P contents of digesta in the gastro-intestinal tract are not well known.

The present experiment was performed to study the effects of pH on availability of Mg, Ca and P in digesta in the gastro-intestinal tract by means of determining pH, Cl^- , HCO_3^- , Mg, Ca, P, Na and K in supernatant fraction of digesta in the gastro-intestinal tract of rats.

MATERIALS AND METHODS

Seven male and female rats of Wistar strain breed, weighing $294 \pm 25\text{g}$, were used. They were fed on commercial pellets (Rabo MR Breeder, Nousan Kagaku Kogyo, Ltd.) and water *ad libitum* in natural environment in June, 1985.

The experiment was carried out during 10:30–15:00. The gastro-intestinal tracts (from the stomach to the colon) of the 7 sacrificed rats were removed and the gastro-intestinal tract were immediately divided into 7 parts: the stomach, 3 segments of about 10 cm of the small intestine (the

Fig. 1 Change of pH in the feed, digesta in the gastro-intestinal tract and feces of rats. **, ***: Significantly different from at least one of the neighboring values at $P < 0.01$ and 0.001 , respectively.

upper, middle and lower segments; S-1, S-2 and S-3), the caecum and 2 segments of about 5 cm of the colon (the upper and lower segments; C-1 and C-2). pH was measured after dilution of digesta with 10.0 ml of distilled water. To determine of the site where pH of digesta changed from acidity (Segment A; S-A) to alkalinity (Segment B; S-B), the segment corresponding to S-2 and S-3 was further divided into 6 strips of 3 cm and the pH of these digesta was measured. The feed and the feces were also collected for analysis.

Feed, digesta and feces were separated into supernatant (Sup) and precipitate (Ppt) by centrifugation at $3,000 \times g$ for 10 min. Samples of Ppt fraction were dried (105°C , 12 hr), ashed (550°C , 12 hr) and dissolved in 1 ml of 6N HCl and 4 ml of distilled water. The pH of each sample was measured with a

Fig. 2 Site of change from acidity to alkalinity of digesta in the small intestine of rats. **: See the legend for Fig. 1.

pH meter. Mg and Ca contents were analysed with an atomic absorption spectrophotometer. P content was analysed by the colorimetric method of Goldenberg [3] and Na and K contents were analysed with a flame spectrophotometer. Cl^- and HCO_3^- contents were analysed by the method of Schales-Schales [11] and the method of Conway [9], respectively.

The results were statistically treated with Student's *t* test and applied to the multiple regression analysis.

RESULTS

Change of pH: The pH in the digesta declined remarkably in the stomach (3.46 ± 0.72) and thereafter rose gradually in S-1 (6.51 ± 0.19), in S-2 (6.86 ± 0.16) and in S-3 (7.99 ± 0.27) and then showed almost constant level, except that the pH in the digesta slightly decreased in the caecum (6.91 ± 0.23) (Fig. 1).

The site where pH changed suddenly (S-A; $6.88 \pm 0.11 \rightarrow$ S-B; 7.40 ± 0.23) in digesta in the small intestine was at the distance of 51.2 ± 6.2 cm from the upper site of the

Fig. 3 Changes of Cl^- and HCO_3^- contents in the supernatant fraction of the feed, digesta in the gastro-intestinal tract and feces of rats.

small intestine (Fig. 2), and that of $74.4 \pm 1.6\%$ for the whole length of the small intestine (68.8 ± 8.3 cm).

Changes in Cl^- and HCO_3^- : Cl^- content was greater in the digesta in the stomach and S-1 than in the feed, but it decreased gradually from S-2 to the caecum and became nearly 0 in the feces (Fig. 3). Cl^- content slightly increased in the digesta in C-1 and C-2.

Though HCO_3^- content was nearly 0 in the feed and the digesta in the stomach, it increased remarkably in the digesta in S-1 and then decreased gradually in the feces. HCO_3^- content transiently increased in the digesta in C-1.

Changes in Na and K: Though Na content in the Sup was greater in the digesta in S-1 and S-2 than that in the feed and in the digesta in the stomach, it decreased suddenly in the digesta in S-B and became almost constant in the segments from S-3 to the feces, except C-2 (0.10 mEq/g D. W.). Na content in the Ppt was greater in the digesta in S-1 than in the feed and in the digesta in the stomach, but it decreased gradually to the feces, except the transient increase in C-1 (0.50 mEq/g D. W.). Sup % of Na was

Fig. 4 Changes of Na and K contents in supernatant and precipitate fractions of the feed, digesta in the gastro-intestinal tract and feces of rats. $\square$: Supernatant fraction. $\blacksquare$: Precipitate fraction.

over 50% (62.5–97.6%) in each sample, except that in C-1 (44.4%) (Fig. 4).

K content in the Sup and Ppt and the Sup % of K showed nearly the same changes as those of Na, except remarkably low Sup % in the digesta in S-1 (27.3%) and in the feces (25.0%).

Changes in Mg, Ca and P: Though Mg content in the Sup was low in the feed and in the digesta in the stomach, it increased remarkably in the digesta in S-1, thereafter decreased gradually and stayed at almost constant levels after S-B, except in C-1 and in the feces. Mg content in the Ppt was greater in the digesta in the stomach than that in the feed, it increased remarkably in the digesta in S-1 and showed a tendency to increase up to the feces, except transient decrease of that in the digesta in the caecum (0.18 mEq/g D. W.). The Sup % of Mg was low in feed (21.4%) but it rose remarkably in the stomach (77.8%) and stayed at almost constant levels up to S-2 (67.2–77.8%) (Fig.

Fig. 5 Changes of Mg, Ca and P contents in supernatant and precipitate fractions of the feed, digesta in the gastro-intestinal tract and feces of rats. □: Supernatant fraction. ■: Precipitate fraction.

5). The Sup % of Mg, however, declined to below 50% in S-B and there was a tendency to decrease gradually in the feces (29.4%), except the transient rise in the caecum (64.7%).

Ca content in the Sup in the digesta, increased gradually from the stomach to S-2, then decreased in S-B and thereafter showed almost a constant level to the feces (0.23–0.33 mEq/g D. W.). Though Ca content in the Ppt of the digesta was less in the stomach and S-1 than in the feed, it increased remarkably in S-2 and thereafter increased gradually to the feces, except a transient increase in the digesta in S-B. Ca content increased gradually from C-1 to the feces (Fig. 5). Though the Sup % of Ca was low in the feed (17.4%), but that in the digesta rose remarkably in the stomach (67.7%) and in S-1 (76.6%) and declined in S-2 (39.3%) and in S-B (9.6%). The Sup % of Ca, however, was at low levels in the samples obtained from the sites from S-3 to

the feces (10.1–16.8%), though it rose a little in the digesta in the caecum (18.2%).

P content in the Sup was low in the feed, increased in the digesta obtained from the sites from the stomach to S-2, decreased and continued at low levels from S-B to the feces (0.08–0.32 mEq/g D. W.). P content in the Ppt of digesta increased gradually up to S-3 and then increased remarkably in the caecum and continued at high levels to the feces (Fig. 5). The Sup % of P in the digesta, which was very low in the feed (8.4%), rose remarkably in the stomach (95.8%), but thereafter it decreased gradually and continued at low levels in the samples obtained from the caecum to the feces (1.2–5.0%).

Correlations among pH, Cl^- , HCO_3^- , Na, K, Mg, Ca and P contents (Table 1): There were significantly positive correlations between Cl^- and HCO_3^- contents, between HCO_3^- and total Na or K contents, and between Na and K contents in the Sup. There were negative correlations between pH and the Sup % of Mg, Ca or P, and positive correlations between Cl^- content and Mg content in the Sup, the Sup % of Ca or the Sup % of P. Furthermore, there were positive correlations between HCO_3^- content and Mg, Ca or P content in the Sup, between Na and Mg, Ca or P contents in the Sup, between total K content and Mg, Ca or P content in the Sup and among the Sup % of Mg, Ca and P.

DISCUSSION

In the present experiment, the pH change of digesta from the stomach to the upper segment of the small intestine (S-1) (3.46→6.51) agreed with that of the values given in a related report [17]. The low H^+ which depended on HCl secretion, in the stomach (Fig. 3) will be neutralized with alkali salts from saliva, pancreatic juice and bile $[\text{2HCl} + \text{NaHCO}_3 + \text{KHCO}_3 \rightarrow \text{2H}_2\text{CO}_3]$

Table 1. Correlations among mean values of pH and Cl^- , HCO_3^- , Na, K, Mg, Ca and P contents in feed, digesta in the gastro-intestinal tract and feces of rats

Item X vs. Y		Regression equation (n=10)		Correlation coefficient
Cl^- (Sup) ^{a)}	vs. HCO_3^- (Sup)	y=	12.912x+ 0.429	0.76701**
HCO_3^- (Sup)	vs. Na(Total) ^{b)}	y=	0.151x+ 0.261	0.90451***
	vs. K(Total)	y=	0.086x+ 0.043	0.96738***
Na(Sup)	vs. K(Sup)	y=	0.314x+ 0.001	0.89450***
pH	vs. Mg(Sup %)	y=	- 11.175x+124.866	-0.65348*
	vs. Ca(Sup %)	y=	- 13.697x+123.477	-0.73668*
	vs. P(Sup %)	y=	- 21.306x+175.927	-0.81836**
Cl^- (Sup)	vs. Mg(Sup)	y=	0.693x+ 0.129	0.67458*
	vs. Ca(Sup %)	y=	85.425x+ 8.713	0.86543**
	vs. P(Sup %)	y=	115.269x+ 1.419	0.83393**
HCO_3^- (Sup)	vs. Mg(Sup)	y=	0.049x+ 0.120	0.80925**
	vs. Ca(Sup)	y=	0.025x+ 0.219	0.71073*
	vs. P(Sup)	y=	0.025x+ 0.219	0.88345***
Na(Sup)	vs. Mg(Sup)	y=	0.293x+ 0.118	0.67557*
	vs. Ca(Sup)	y=	0.234x+ 0.169	0.93005***
	vs. P(Sup)	y=	0.879x+ 0.015	0.86577**
K(Total)	vs. Mg(Sup)	y=	0.518x+ 0.114	0.75613*
	vs. Ca(Sup)	y=	0.257x+ 0.218	0.64605*
	vs. P(Sup)	y=	1.405x+ 0.053	0.87605***
Mg(Sup %) ^{c)}	vs. Ca(Sup%)	y=	0.740x- 6.667	0.68039*
	vs. P(Sup %)	y=	1.118x- 24.983	0.73436*
Ca(Sup %)	vs. P(Sup %)	y=	1.326x- 9.673	0.94676***

a) Content in supernatant fraction in each sample (mEq/g D. W.).

b) Total content in each sample (mEq/g B. W.).

c) Sup to Total contents $\times 100$.*, **, ***: $P < 0.05$, 0.01 and 0.001, respectively.

+NaCl+KCl], because the content of HCO_3^- , Na and K in digesta increased in S-1 (Figs. 3 and 4). The contents of Cl^- , HCO_3^- , Na and K in digesta gradually decreased from S-1 to the middle segment of the small intestine (S-2), and the decrease may be caused by absorption in the small intestine (Figs. 3 and 4). The pH in digesta changed from weak acidity (pH6.88) to alkalinity (pH7.40) at the defined site in the small intestine (between S-A and S-B) which was at the distance of about 75%

from the upper site of the small intestine (Fig. 2). Beyond the site, the pH of digesta gradually rose in alkalinity (pH7.40–7.99). These changes in pH might be due to the dissociation of H_2CO_3 into H_2O and CO_2 [$2\text{H}_2\text{CO}_3 + \text{NaHCO}_3 + \text{KHCO}_3 \rightarrow 2\text{H}_2\text{O} + 2\text{CO}_2 \uparrow + \text{NaHCO}_3 + \text{KHCO}_3$], because HCO_3^- , Na and K contents remained constant after S-B (Figs. 3 and 4). On the other hand, pH of digesta in the caecum returned to weak acidity (pH6.91) (Fig. 1). This acidification might be due to organic acids

(lactic acid, volatile fatty acids, etc.) which were produced by fermentation in the caecum [14]. From the upper segment (C-1) in the colon to the feces, the pH of digesta again changed in alkalinity (pH7.75–7.97) (Fig. 1). Hydrogen ions of organic acids might be neutralized by intestinal juice secreted in the upper site of the colon, since Cl^- , HCO_3^- , Na and K contents increased in C-1. But it has not been confirmed that the intestinal juice contained alkali salts secreted in the colon of the rat. The changes in pH was not directly related with those of Cl^- , HCO_3^- , Na and K contents in the samples obtained from the feed, digesta in the gastro-intestine and feces. This may be the results of the neutralization of H^+ , the dissociation of HCO_3^- and the absorption of Cl^- , Na^+ and K^+ . However, some relationship between pH and Cl^- , HCO_3^- , Na and K contents of digesta were suggested by high negative correlations between them in digesta of the small intestine ($P < 0.1$, respectively) (Table 1).

In the present experiment, Mg, Ca and P contents of digesta in the Ppt fractions were more affected by pH than the Sup % of Na and K contents according to the differences of the ionization tendency of these minerals. Though the Sup % of Mg, Ca and P declined as pH rose, the decreasing rate was lower in an order of Mg, Ca and P (Fig. 5 and Table 1). Generally, hydrogenphosphate and phosphate ions (HPO_4^{2-} and PO_4^{3-}) easily combine with Mg^{2+} or Ca^{2+} to form insoluble phosphate [MgHPO_4 and $\text{Mg}_3(\text{PO}_4)_2$ or CaHPO_4 and $\text{Ca}_3(\text{PO}_4)_2$] as pH rises, especially in alkalinity [10]. The formation of phosphates might be much less in Mg than in Ca in the gastro-intestinal tract of rats (Fig. 3 and Table 1), because the ionization tendency of Mg is smaller than that of Ca.

It is reported that Mg is absorbed over a wide range from the duodenum to the colon [4, 5, 7, 8, 15] mainly by diffusion [2, 5]. On

the other hand, Ca is absorbed in limited sites in the duodenum [18] and ileum [12], mainly by active transport [20]. It is also reported that Mg and Ca in digesta compete at the absorption site of the small intestine [1, 6]. Thus, it is generally accepted that the difference between Mg and Ca (Fig. 5 and Table 1) contents depends on difference in absorption sites or absorbing mechanisms. Our results showed that their physico-chemical status of Ca and Mg in the gastro-intestinal tract is also important in the regulation of absorption of them. The marked increase of P precipitate of digesta in the caecum might be due to the accumulation of P (phospholipids, phosphoprotein, etc.) into bacteria in the caecum [14, 17].

These results suggested that pH played important roles in regulating absorption of Mg, Ca and P by changing the physico-chemical properties of digesta through the gastro-intestinal tract of rats.

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要 約

ラット胃・腸管内容物の pH と Mg, Ca, P, Na, K, Cl^- および HCO_3^- 含量の相互関係：志賀隴郎・佐々木剛・堀井菜摘子（岩手大学農学部獣医学科家畜生理学教室）——7頭のラットについて、飼料、胃、小腸4部位（上部、中部、内容物のアルカリ性転換部（S—B）、および下部）、大腸3部位（盲腸、結腸上部および下部）の各内容物および糞の pH, pH の変化と関連する Cl^- , HCO_3^- , Na および K 含量ならびに Mg, Ca, P 含量を測定し、以下の成績を得た。内容物の胃での酸性化（pH 7.10→3.46）、小腸上部での pH の急上昇（pH 3.46→6.60）、S—B での急激なアルカリ化（pH 6.86→7.40）、盲腸での酸性化（pH 7.97→6.91）、結腸上部でのアルカリ化（pH 6.91→7.97）が認められた。S—B は、小腸開始部から約75%の部位であった。 Cl^- , HCO_3^- , Na, K 含量は、pH との間に相関はなかったが相互に正の相関があり、胃での HCl 分泌、盲腸での有機酸発酵に対応し、ミネラルの中和調節効果が示唆された。Mg, Ca, P の可溶性含有率（Sup %）は相互間に正の相関があり、それぞれが pH との間に負の相関をもち、pH の上昇に伴う Mg, Ca, P の沈殿促進効果が認められた。盲腸での P 含量の急増から、微生物体への P の蓄積が示唆された。