Transport of Calcium, Phosphorus and Magnesium Across the Rumen Wall

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Abstract In conscious sheep, the transport of calcium, phosphorus and magnesium across the rumen to the blood was observed using the method of veno-arterio differences. Two wethers, in individual pens, were each surgically prepared with a rumen fistula, a carotid loop and a rumen venous catheter and they were fed a diet of barley, soybean meal and hay. Blood and rumen fluid samples were taken 6 times a day as follows, 0, 1.0, 1.5, 2.5, 4.0 and 7.0 hours after feeding. The absorption and secretion of calcium, phosphorus and magnesium was demonstrated in normal mineral concentrations and pH values in the rumen fluid. The considerable amount of calcium and phosphorus were transported through the rumen wall. But the net adsorption of calcium and phosphorus was small in quantity. A positive correlation between the veno-arterio differences of calcium and phosphorus was found in one sheep ($P < 0.5$). Magnesium seemed to be absorbed constantly across the rumen wall when the rumen fluid magnesium levels averaged 100 mg/l. Noticeable correlation was not found between the magnesium absorption levels and the rumen magnesium concentrations, and the rumen pH values.

It is known that the ruminal epithelium is similar to the frog skin epithelium in having a selective permeability¹⁾. The exchanges of sodium, potassium, chloride and water through the rumen wall have been elucidated by many researchers²⁻⁷⁾, whereas the reports on the transportation of calcium, phosphorus and magnesium through the rumen wall have been controversial.

Using the marker ratio technique, SMITH *et al.*⁸⁾, CHANDLER and CRAGLE⁹⁾, YANG and THOMAS¹⁰⁾ and PFEFFER *et al.*¹¹⁾ elucidated the absorption or secretion of calcium and phosphorus from the rumen. And, GRACE and MACRAE¹²⁾, BEN-GHEDALIA *et al.*¹³⁾ and HORN and SMITH¹⁴⁾ reported the magnesium absorption from the sites of reticulo-rumen and omasum. TSUDA¹⁵⁾, CARE and VAN'T KLOOSTER¹⁶⁾ and PHILLIPSON and STORRY¹⁷⁾ concluded, by the rumen pouch and isolated rumen sac techniques, the rumen epithelium was relatively impermeable to calcium, phosphorus and magnesium.

MOODIE and WALKER¹⁸⁾ reported on the mineral absorption from the digestive tract of sheep by comparing the composition of arterial and portal venous blood. Total quantity of mineral absorbed from all of the absorption sites could be examined but the transport of minerals across the each absorption site could not be estimated in their experiment.

The purpose of the present report is to study the absorption or secretion of calcium, phosphorus and magnesium through the rumen wall in conscious sheep using the method of ruminal veno-arterio differences.

Materials and Methods

Animals

Two adult wethers, 54 and 34 kg in body weight, were used. These animals were each fitted with a rumen fistula¹⁹) and a carotid loop²⁰). They were kept in individual pens and given a diet of concentrate and hay (Table 1).

Table 1. Composition of basal diet.

Ingredient	Content (%)
Barley	30
Soy bean meal	10
Orchard grass (hay)	60

Surgical preparation of animals

The animals were starved for 24 hours before the surgical operation. The sheep were placed in right lateral recumbency. Combelen (phenothiazine) was administered intramuscularly 15 minutes before the sheep was anesthetized. Then the animal was anesthetized via the jugular vein with nembutal (pento barbiturate sodium).

A skin incision about 20 cm long was made parallel to the last right rib. The rumen and its right blood vessels were exposed and two ligatures were placed around the branch of the right ruminal vein which was separated from the rumen surface and the ligatures were left not tightened. A small opening sufficient to accomodate the tubing was made in the venous wall. A silicized catheter (Fr. 5, 60 cm long, Medical tube, Dow Corning Co.) was inserted to the lumen of the vein and the ligatures were tightened with the vessel (Figure 1).

Fig. 1. Right surface of rumen and position of venous catheter.

The catheter was filled with heparin solution in saline (500 IU/ml) and the free end of the catheter was closed with an obturator and fixed to the abdominal skin by adhesive. After surgery, the catheter was flushed at 4-12 hours intervals. When the catheter was open and blood was withdrawn, the catheter was flushed and filled with the heparin saline solution.

Experimental methods

On the second day after the operation, blood samples of the ruminal vein were obtained from the catheter. The samples were collected at 0, 1.0, 1.5, 2.5, 4.0 and 7.0 hours after feeding. Arterial blood was taken from the carotid loop by an injecting syringe and rumen fluid was removed through the rumen fistula at the same time. These samples were taken from one sheep for 4 days and the other for 2 days.

The hemoglobin content was determined²¹⁾ to correct the fluctuation of water content in both arterial and venous bloods due to absorption of water from the digestive tract and secretion from the salivary glands. Blood serum was then separated and stored at -14°C . The pH value of rumen fluid was measured with pH test paper and centrifuged for 30 minutes at 12,000 rpm. and the supernatant was removed and stored till analyzed.

The determination of serum magnesium concentration was carried out in triplicate and the other mineral estimations in duplicate. The coefficients of variation of replicates in samples were within 2-5% for three elements. The paired arterial and venous samples were analyzed simultaneously. The concentrations of calcium and magnesium were measured by an atomic absorption spectrophotometry and inorganic phosphorus by the method of FISKE-SUBBAROW²²⁾.

Results

Calcium concentrations in rumen fluid and the veno-arterio differences are shown in Table 2. Rumen calcium concentrations fell into a range from 60 to 100 mg/l. The serum calcium was about 80 mg/l in sheep 1 and about 100 mg/l in sheep 2. These values were within normal levels

Table 2. Calcium veno-arterio differences and calcium concentrations of rumen fluid, rumen venous serum and carotid serum for sheep. (mg/l)

Sheep No.	Time after feeding	Rumen fluid	Ruminal Vein ^a	Carotid Artery	Ruminal Vein ^a - Artery (range)
I ^b	0	68	82.7	78.7	4.0 (- 1.6 ~ 10.6)
	1.0	66	82.2	83.6	-1.4 (- 5.5 ~ 1.4)
	1.5	74	81.5	82.3	-0.8 (- 6.2 ~ 1.5)
	2.5	109	80.4	80.6	-0.2 (- 2.7 ~ 1.9)
	4.0	118	81.3	82.6	-1.3 (- 3.6 ~ 1.9)
	7.0	100	79.5	85.0	-5.5 (- 11.2 ~ - 2.7)
II ^c	0	62	102.9	99.0	3.9 (3.9)
	1.0	86	106.9	105.1	1.8 (- 4.8 ~ 8.4)
	1.5	105	100.5	97.5	3.0 (0.6 ~ 5.3)
	2.5	100	107.2	99.1	8.1 (1.6 ~ 14.6)
	4.0	93	105.5	99.4	6.1 (2.5 ~ 9.6)
	7.0	67	101.0	99.9	1.1 (1.0 ~ 1.2)

a: Corrected values by veno-arterio hemoglobin ratio. b: Means of four days' samples. c: Means of two days' samples.

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Table 3. Phosphorus veno-arterio differences and phosphorus concentrations of rumen fluid, rumen venous serum and carotid serum for sheep. (mg/l)

Sheep No.	Time after feeding	Rumen fluid	Ruminal Vein ^a	Carotid Artery	Ruminal Vein ^a - Artery	(range)
I ^b	0	1161	59.6	57.8	1.8	(- 4.5~ 10.5)
	1.0	1093	59.0	61.3	-2.3	(- 9.2~ 2.1)
	1.5	1087	61.5	62.6	-1.1	(- 4.2~ 0.9)
	2.5	1053	60.3	62.1	-1.8	(- 8.5~ 2.9)
	4.0	1097	57.5	57.3	0.2	(- 1.9~ 3.3)
	7.0	1128	52.1	52.6	-0.5	(- 1.3~ 1.0)
II ^c	0	906	60.2	66.0	-5.8	(- 5.8)
	1.0	811	71.2	68.3	2.9	(2.0~ 3.8)
	1.5	831	70.2	64.0	6.2	(6.2)
	2.5	875	52.9	54.4	-1.5	(- 4.4~ 1.3)
	4.0	829	48.5	44.4	4.1	(4.1~ 4.2)
	7.0	775	50.1	47.7	2.4	(2.4~ 2.5)

a: Corrected values by veno-arterio hemoglobin ratio. b: Means of four days' samples. c: Means of two days' samples.

for sheep^{18,23}. The veno-arterio differences for calcium showed ranges from -11.2 to +10.6 mg/l in sheep 1 and from -4.8 to +14.6 mg/l in sheep 2. The results might indicate that a considerable amount of calcium was absorbed and/or secreted through the rumen wall. In sheep 1, calcium absorption was recognized before feeding and secretion after feeding. However, almost in all cases, the net calcium absorption was found in sheep 2. A definite trend with the sampling time was not observed.

The results of phosphorus are shown in Table 3. Rumen phosphorus concentration was about

Table 4. Magnesium veno-arterio differences and magnesium concentrations of rumen fluid, rumen venous serum and carotid serum for sheep. (mg/l)

Sheep No.	Time after feeding	Rumen fluid	Ruminal Vein ^a	Carotid Artery	Ruminal Vein ^a - Artery	(range)
I ^b	0	49	25.4	22.6	2.8	(1.3~ 5.2)
	1.0	50	24.1	23.8	0.3	(- 0.3~ 1.5)
	1.5	63	24.4	22.7	1.7	(0.3~ 3.8)
	2.5	74	24.5	23.0	1.5	(0.9~ 2.1)
	4.0	72	24.6	24.4	0.2	(- 1.1~ 1.7)
	7.0	64	25.2	23.5	1.7	(- 0.2~ 3.5)
II ^c	0	41	24.7	22.6	2.1	(2.1)
	1.0	98	28.1	25.3	2.8	(2.3~ 3.1)
	1.5	119	25.7	24.1	1.6	(1.6~ 1.7)
	2.5	110	26.0	25.0	1.0	(0.6~ 1.3)
	4.0	103	29.7	26.0	3.7	(2.1~ 5.3)
	7.0	51	27.6	26.4	1.1	(0.5~ 1.7)

a: Corrected values by veno-arterio hemoglobin ratio. b: Means of four days' samples. c: Means of two days' samples.

1,100 mg/l and 850 mg/l in each sheep. There were some differences between the two animals, but these phosphorus values in rumen fluid of sheep were within normal range²³). The serum phosphorus levels were from 50 to 70 mg/l and within normal concentration¹⁸). The value of veno-arterio differences for phosphorus ranged from -9.2 to +10.5 mg/l in sheep 1 and from -5.8 to +4.1 mg/l in sheep 2. The results indicated that phosphorus might be absorbed and/or secreted through the rumen wall. There was no distinct trend for phosphorus transport after feeding. A significant correlation was observed between the veno-arterio differences of calcium and that of phosphorus in sheep 1 ($r=0.449$, $P<.05$).

Magnesium concentrations in sheep are indicated in Table 4.

Rumen magnesium concentrations were about 40-120 mg/l. Serum magnesium levels were from 20 to 26 mg/l in sheep 1 and from 22 to 29 mg/l in sheep 2. These were within normal range for sheep^{18,23}). The veno-arterio differences for magnesium were from -1.1 to +5.2 mg/l in sheep 1 and from +0.5 to +5.3 mg/l in sheep 2. However, no definite tendency in veno-arterio differences for magnesium was found with time after feeding.

Discussion

Some researchers^{24,25}) reported that calcium and magnesium solubilities are enhanced by the lowering of rumen pH. While it has not been clarified whether rumen pH values affect the transport of calcium and magnesium through the rumen wall or not. Veno-arterio differences for calcium, phosphorus and magnesium had almost no relationship to rumen pH in this experiment.

PHILLIPSON and STORRY¹⁷) demonstrated the rumen epithelium appeared to be relatively impermeable to calcium. On the other hand, YANG and THOMAS¹⁰) showed more calcium was absorbed than secreted in reticulo-rumen. PFEFFER *et al.*¹¹) observed the small gains in calcium in digesta flowing through the reticulo-rumen. In the present experiment, the mean value of the veno-arterio differences for calcium was about -0.9 mg/l in sheep 1 and about +4.5 mg/l in sheep 2. Results would indicate the possibility of calcium transport through the rumen wall even if the net absorption or secretion was small in quantity. In this connection, STORRY²⁶) reported that rumen calcium concentrations were insufficient for absorption through the rumen wall. However, in this experiment, the transport of calcium seemed to occur although rumen calcium levels fell into normal range.

SCARISBRICK and EWER²⁷) found that absorption of in-organic phosphate from the rumen was possible. SMITH *et al.*⁸) stated that a significant amount of phosphorus came by way of saliva but a larger amount came through the rumen wall. Later, DOBSON²⁸) reported that the rumen epithelium was impermeable to phosphorus. The data obtained in the present experiment indicated the occurrence of the absorption and secretion of phosphorus through the rumen wall. The maximum values of phosphorus absorption and secretion were +10.4 mg/l and -9.2 mg/l, respectively. That is, a large amount of phosphorus, about 15% of blood serum concentration, would be transported through the rumen wall. Serum phosphorus concentrations were very lower than those of rumen fluid, about 1/20 in levels, while the result may suggest that the serum phosphorus was sometimes secreted into the rumen against the gradient.

STORRY²⁶) and PHILLIPSON and STORRY¹⁷) reported that the rumen epithelium was impermeable to magnesium in sheep. And, CARE and VAN'T KLOOSTER¹⁶) demonstrated that net magnesium absorption from the gastric pouches was little in sheep and that magnesium ions would only move in the rumen-blood direction when the intraruminal ionic magnesium was more than ten times greater (17 m-equiv./l) than the plasma ionic magnesium concentration. But, PFEFFER *et al.*¹¹)

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GRACE and MACRAE¹²⁾ and HORN and SMITH¹⁴⁾ reported that magnesium was absorbed from the stomach in the ruminant. And TOMAS and POTTER²⁹⁾ showed the important site of magnesium absorption was the rumen. From the result that there were veno-arterio differences of magnesium in both sheep, it was considered that magnesium was absorbed or secreted through the rumen wall. However, magnesium seemed to be absorbed in most cases because mean values of veno-arterio differences of magnesium was $+1.4 \text{ mg/l}$ for sheep 1 and $+2.0 \text{ mg/l}$ for sheep 2. Furthermore, magnesium concentrations of rumen fluid were within normal range. Therefore, the rumen may be a significant absorption site of magnesium as suggested by TOMAS and POTTER²⁹⁾. But the rumen magnesium concentrations and the rumen pH values did not appear to affect magnesium absorption from the rumen. This finding differed from the results by HORN and SMITH¹⁴⁾.

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第一胃壁からのカルシウム、リンおよびマグネシウムの輸送

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反すう動物の第一胃からのカルシウム、リン、マグネシウムの吸収・分泌を検討するために、2頭の去勢メン羊を用い、動静脈血差法による試験を実施した。メン羊は、第一胃フィステル、頸動脈ループおよび第一胃静脈カテーテルが取り付けられ、大麦、大豆粕、乾草が1日2回給与された。朝の給餌前(0時間)、給餌終了後(1時間)、以下1.5, 2.5, 4.0, 7.0時間目の1日計6回、メン羊1で4日間、メン羊2では2日間にわたり第一胃内容液、動脈血および第一胃静脈血が採取された。その結果、第一胃内容液中の各ミネラル含量が通常の場合でも、カルシウム、リン、マグネシウムの第一胃壁からの

吸収・分泌が認められ、第一胃壁はカルシウム、リン、マグネシウムに対して透過性を有することが示された。マグネシウムは、第一胃内容液中のマグネシウム含量が100 mg/l 前後にある時も、常時吸収されているような傾向を示した。飼料摂取後の経時的な変化には、各ミネラルともに一定の傾向はなかった。また、従来、関係があるとされていたマグネシウム吸収量と、第一胃内容液マグネシウム含量、および第一胃内容液 pH 値の間には、本試験の結果からは有意な相関は認められなかった。