The Effect of Bilateral Ligation of the Parotid Duct on Phosphorus, Sodium and Potassium Metabolism and Acid-Base Balance in the Goat

Hiroshi SATO

Department of Animal Physiology, National Institute of Animal Industry, 959 Aoba-cho, Chiba-shi, Chiba 280

(Received for publication August 26, 1974)

Abstract. 1. The bilateral parotid ducts were ligated in five goats to study the influence of parotid salivation on phosphorus, sodium and potassium homeostasis and acid-base balance. The compositions of urine, feces, blood and rumen fluid were determined during the control period and for four weeks after ligation.

2. The parotid duct ligation caused an increase in urinary phosphorus excretion and plasma inorganic phosphorus level. Fecal phosphorus output decreased, but total phosphorus output in urine and feces changed little. It seems likely that the increase in plasma and urinary phosphorus level and the decrease in fecal phosphorus output may have been caused by the decrease in phosphorus recycling via parotid salivation. Urine pH became acidic and urinary titratable acid excretion increased after ligation.

3. Urinary sodium and chloride excretion decreased transiently by ligation and then returned to the initial level. Urinary potassium excretion did not change after ligation, except for a few days. Fecal sodium and potassium output was not affected.

4. Sodium and inorganic phosphorus levels of rumen fluid decreased rapidly by ligation and then tended to return to the initial levels. Duct ligation caused a small increase in potassium level and little change in pH of rumen fluid.

5. Plasma bicarbonate, sodium, potassium and chloride levels and blood pH scarcely changed.

6. In ruminants, phosphorus homeostasis seemed to have a close correlation to the parotid function, since a decrease in parotid salivation caused a change in phosphorus homeostasis. It was assumed, however, that sodium and potassium homeostasis might not be influenced so much by the ligation of the parotid duct.

In monogastric animals, such as man and dog, a large amount of phosphorus is excreted in urine, and the kidney plays a major role in regulation of phosphorus balance. On the other hand, urine of the ruminant normally contains a small amount of phosphorus and shows an alkaline reaction [16, 19, 22, 23]. Thus, Young et al. [31] reported that the phosphorus balance of the ruminant was regulated more efficiently by the intestine than by

the kidney.

A ruminant fed a high energy diet, however, exhibited phosphaturia and a decline in urinary pH [17, 29]. The reason for this is not known. Scott [20] suggested that the salivary phosphorus excretion might decrease according to the reduced saliva flow caused by concentrate feeding. Recently, Tomas et al. [26] and Tomas [27, 28] showed that the bilateral ligation of parotid ducts caused an increase in urinary

ヤギにおける両側耳下腺管の結紮がリン、ナトリウム、カリウムの代謝および酸-塩基平衡に及ぼす影響： 佐藤 博（農林省畜産試験場）

phosphorus excretion and in plasma phosphorus level in sheep, and that an increase in phosphorus excretion in urine was caused by feeding finely ground roughage.

The parotid saliva of the ruminant contains large amounts of sodium [11, 12] and bicarbonate [11]. The influence of parotid duct ligation on sodium and potassium homeostasis and acid-base status, however, is yet unknown. The experiment described in this paper was designed to clarify the influence of parotid duct ligation on the homeostasis of phosphorus, sodium, and potassium and acid-base balance in goats.

Materials and Methods

1. Animals and diets: Five Japanese native male goats (Nos. 51, 52, 520, 521 and 740) weighing from 19 to 36 kg were used. In three of them, a fistula was attached to the rumen. Equal amounts of concentrate and alfalfa hay cube were given every morning (600–940 g daily). They were allowed to take water *ad libitum*. Feed and water intake were measured daily. Mineral contents of the diets are given in Table 1. Each animal was accustomed to a metabolic cage for one month before the experiments was begun. At the end of the experiment, each animal was autopsied. The experiment was performed between October, 1973 and May, 1974.

2. Ligation of the parotid duct: Under pentobarbital anesthesia, the skin was incised at a level 1–2 cm above the incisura vasorum, the duct and nerves were exposed above the masseter muscle, and the duct was separated from the nerves as far as possible and tied with silk thread at two sites. For several days after operation, procaine penicillin was injected intramuscularly every day.

3. Sample collection: Urine samples were collected from each animal before feeding in the morning for four days before ligation (control period), for the first 14 days and from 25 to 28 days after ligation into a polyvinyl bucket containing 10 ml of toluene. Urine volume, pH, and titratable acid and base were measured quickly, and a part of the urine collected was frozen until analysis.

Fecal samples were collected from three goats during the control period, from 25 to 28 days after ligation, and from one of these goats daily for the first 14 days after ligation. They were dried at 60°C for 24 hours. The air-dried samples were weighed.

Table 1. Mineral contents of diets used
(% value on air dry basis)

	Ash	Na	K	P
Concentrate	5.84	0.46	0.66	0.62
Hay cube	7.84	0.18	1.98	0.17

Some of them were stored for analysis.

Blood samples were taken from five goats by jugular puncture into heparinized tubes immediately before feeding in the control period and 3, 7, 11, 14, 24 and 28 days after ligation. Blood plasma was separated and stored. In two animals, blood was sampled under liquid paraffin for blood pH and plasma bicarbonate analysis.

In three animals with a rumen fistula fitted, samples of ruminal digesta were taken immediately before feeding when blood samples were collected, and strained through a double layer of gauze. Then they were centrifuged at 2500 G for 30 minutes and the resulting supernatant was frozen.

4. Analytical method: Titratable urinary acid and base were measured in the same manner as described by Henderson et al. [9]. Urinary and rumen fluid pH was determined by a pH meter, and blood pH by Cullen's method [5]. Plasma bicarbonate was determined by Natelson's microgasometer, and phosphorus by Fiske-Subbarow's method [7]. Plasma and urinary chloride was determined by Schales and Schales's method [18]. Sodium and potassium were analyzed by flame photometry. Feces and diets were prepared for analysis by ashing 3 g of sample in the muffle furnace at 600°C for 24 hours. The ash was taken up with 6N hydrochloric acid and diluted to 250 ml with deionized water. Then sodium, potassium, and phosphorus contents were estimated.

Results

1. Feed intake and body weight: Two animals refused a small amount of concentrate on the next day of ligation, but the others left no residue of feed. All the animals gained in live weight during the experimental period, except one, No. 520, which lost about 2 kg. Successful duct ligation was confirmed by autopsy.

2. Water intake and urine volume: In all the animals, except one, water intake and urine volume were reduced temporarily

Fig. 1. Changes in urine pH

Remarks.

- Goat 51
- Goat 52
- Goat 520
- Goat 521
- Goat 740

The same symbols are used in Fig. 3.

Fig. 2. Urinary excretion of inorganic P, Na and K

Remarks. The value is given as mean $\pm$ S.E.

ly by duct ligation, but increased to the initial level within two weeks after ligation. In goat No. 52, however, little changes were seen after ligation.

3. Urine pH and titratable acid-base excretion: In the control period, two animals (No. 52 and 521) excreted 100 to 150 mEq of titratable base in urine. Their

Table 2. Changes in plasma electrolyte level and blood pH

	Control period	Time in days after ligation					
		3	7	11	14	24	28
Inorganic P (mg/100 ml)	7.4 (1.9)	8.9 (1.9)	9.3 (2.3)	9.5 (2.4)	8.5 (2.1)	9.6 (2.0)	10.4* (1.2)
Na (mEq/l)	139.9 (3.4)	139.9 (3.3)	139.2 (2.9)	138.8 (2.9)	139.7 (3.9)	139.2 (4.3)	139.3 (2.6)
K (mEq/l)	4.0 (0.3)	3.9 (0.3)	4.1 (0.1)	4.1 (0.3)	3.8 (0.2)	4.1 (0.1)	4.0 (0.3)
Cl (mEq/l)	106.8 (3.0)	106.5 (1.7)	105.7 (3.7)	106.0 (2.7)	107.2 (3.7)	108.5 (3.7)	106.6 (2.3)
HCO ₃ ⁻ (mEq/l)	28.7	27.2	28.1	27.2	27.6	26.7	28.3
Blood pH	7.34	7.35	7.37	7.38	7.39	7.39	7.38

Remarks.

In parenthesis is shown the standard error (n=5).

The values for HCO₃⁻ and blood pH are the means of two goats.

* Significant at P<0.05.

urinary pH was alkaline (pH 8.30–8.52). In the other animals, urine pH was 7.20 to 8.00 and titratable acid-base excretion was nearly negligible.

In all the animals, except goat No. 52, duct ligation caused a decrease in urine pH and an increase in titratable acid excretion in urine. On an average, titratable acid in urine increased by 184.4 mEq (daily) in a few days after ligation and by 78.2 mEq (daily) in 25–28 days after ligation compared with that of the control period.

4. Urinary phosphorus, sodium, potassium and chloride excretion: In each animal, urinary phosphorus excretion increased after duct ligation (Fig. 2). Especially 1–4 days after ligation, urinary phosphorus excretion increased to be 1.4 to 21 times as much as that in the control period. When its average value was calculated in the five goats for four consecutive days, urinary phosphorus excretion was 0.53, 1.31 and 1.05 g/day for the control period, a period of 1 to 4 days, and a period of 25 to 28 days after ligation, respectively. Uri-

nary phosphorus concentration was 16.9 mg/100 ml for the control period, 78.4 mg/100 ml for the period of 1 to 4 days (significant increase at P<0.05), and 40.4 mg/100 ml for the period of 25 to 28 days after ligation. There was a large individual difference in urinary phosphorus excretion. Daily phosphorus excretion fluctuated from 0.016 to 1.72 g/day in the control period.

Urinary sodium excretion decreased in each animal after duct ligation. It continued to decrease for a few to about ten days and then increased to the initial level. It seems that the changes in water intake and urine volume may have had no effect on urinary sodium excretion. Urinary potassium excretion increased slightly for the first two days after ligation, but changed little thereafter (Fig. 2). Urinary chloride excretion was parallel with urinary sodium excretion. Urinary potassium concentration increased slightly for a few days after ligation, and then decreased to the initial level. Sodium and chloride concentration were hardly influenced by duct

Fig. 3. Changes in pH, inorganic P, Na and K level of rumen fluid sampled before feeding

ligation.

5. Blood and plasma constituents (Table 2): Duct ligation caused an increase in the inorganic phosphorus level of plasma in each animal. In goat No. 52, the increased plasma phosphorus level declined transiently over a period from 11 to 14 days after ligation, and then increased again. In the other goats, this increase continued for four weeks.

6. Rumen fluid constituents (Fig. 3): The inorganic phosphorus and sodium levels of rumen fluid declined markedly after duct ligation. When its average value was calculated in three animals, inorganic phosphorus level decreased from 168.9 to 66.5 mg/100 ml in 3 days after ligation, and sodium level from 109.6 to 90.8 mEq/l in 3 days and to 85.7 mEq/l in 7 days after ligation. These constituents tended to increase thereafter, but failed to return completely to the initial value within four weeks. Potassium showed a slight elevation in level, and pH changed little.

7. Ratios of urinary to fecal output of

phosphorus, sodium and potassium (Table 3): During the control period, fecal phosphorus output was 53–99% of total phosphorus output in urine and feces. Parotid duct ligation brought about an apparent decrease in fecal phosphorus output in two goats in which urinary phosphorus excretion was elevated by ligation. In goat No. 52, a very small amount of phosphorus was excreted in urine during the control period, and duct ligation had just a little effect on urinary phosphorus excretion. Consequently, it is considered that this animal may be a specific case in phosphorus excretion. From goat No. 51, fecal samples were also collected for the first 14 days after ligation daily. They revealed that the ratio of fecal to urinary output of phosphorus might be stabilized about 12 days after ligation.

Fecal sodium and potassium output were small in amount. The ratio of fecal to total (urinary + fecal) output was 4.7–14.4% for sodium, and 1.8–5.9% for potassium. Duct ligation had little effect on the

Table 3. Ratios of urinary to fecal output of P, Na and K (g/day)

Goat No.	Period	Body weight (kg)	Feed intake		(P)			Na			K		
			Concentrate	Hay cube	Feces	Urine	Total	Feces	Urine	Total	Feces	Urine	Total
51	C*	19.5	300	300	1.34	0.29	1.63	0.12	1.32	1.44	0.27	7.59	7.86
	L**	21.5	300	300	0.82	0.75	1.57	0.22	1.31	1.53	0.45	7.49	7.94
52	C*	25.0	400	400	2.68	0.02	2.70	0.16	1.34	1.50	0.21	10.03	10.24
	L**	26.0	400	400	2.41	0.04	2.45	0.14	1.51	1.65	0.19	10.52	10.71
520	C*	30.1	470	470	1.96	1.72	3.68	0.15	2.89	3.04	0.58	10.99	11.57
	L**	27.8	470	470	1.35	2.29	3.64	0.17	3.45	3.62	0.57	9.12	9.69

Remarks.

C*: Control period (for 4 days).

L**: A period of 25 to 28 days after ligation (for 4 days).

pattern of sodium and potassium output. In goat No. 520, there was a large variance between the control period and the period of 25 to 28 days after ligation in the amount of urinary sodium and potassium excretion. Consequently, it must be considered that this goat have lost in body weight during the experimental period, and that these data were obtained for a short period (4 days) of sampling.

Discussion

The presence of an interrelationship between phosphorus homeostasis and parotid function in the ruminant was demonstrated by the fact that parotid duct ligation caused phosphaturia. Generally, the parotid saliva of the ruminant contains a large amount of phosphorus [2, 10, 12] and gives a favorable circumstance to rumen microbes. The inorganic phosphorus level of parotid saliva was reported to be 15 times [12] and 6.6 to 13 times [24] as high as that of serum. Tomas et al. [24] mentioned that in sheep given four levels of dietary phosphorus (0.42–4.02 g/day), total salivary phosphorus flow had ranged from 3.0 g/day on the lowest dietary phosphorus

to 5.3 g/day on the highest, and that its variation had been less than two times. This indicates a more effective recycling of phosphorus via the salivary glands of a ruminant fed a low phosphorus diet.

Clark et al. [4] emphasized the importance of parotid function in phosphorus homeostasis. It is assumed that the increase in the 'blood (plasma) pool' of phosphorus may have caused an increase in urinary phosphorus excretion by reducing phosphorus inflow into the alimentary tract via the salivary glands. In goat No. 52, urinary phosphorus excretion and plasma inorganic phosphorus level increased concurrently for some time after duct ligation, and decreased in the second week after ligation, but increased again together. This suggested that the increase in plasma phosphorus level might be related to phosphaturia. Tomas [27] showed, however, that sheep given finely ground roughage had excreted more phosphorus in urine than sheep fed oat hulls or polyethylene flakes, but that neither plasma inorganic phosphorus level nor glomerular filtration rate (GFR) changed in the former sheep. In another report of his [28], he explained

that the increase in urinary phosphorus excretion was attributed partly to the increased plasma phosphorus level and partly to the reduced efficiency of renal tubular reabsorption of phosphorus by restricted phosphorus recycling via the parotid glands.

As shown in Table 2 and Fig. 2, urinary phosphorus excretion increased rapidly in a few days after ligation, though plasma inorganic phosphorus level increased slowly. It is likely that the tubular reabsorption of phosphorus may have been adapted to ligation progressively, and that a very large amount of filtered phosphorus may have been excreted in urine in the early period after duct ligation.

It seems that parotid duct ligation may have an effect on animals acutely and chronically. In the early period after ligation, the effect of surgery per se cannot be disregarded, and mineral excretion in urine fluctuated from day to day. It is considered that over a period of 25 to 28 days after ligation, the effect of surgery per se may have disappeared, and that an adaptive change to the stopped parotid inflow may have been brought about in compensation. Therefore, it is suitable to use the data obtained from the period of 25 to 28 days after ligation for approaching studies on the mineral metabolism of a ruminant fed a concentrate diet.

Duct ligation caused an increase in urinary phosphorus excretion, but nearly the same amount of phosphorus decreased in fecal output, and slight changes occurred to total output in urine and feces. So far as urinary pH and phosphorus excretion are concerned, it is considered that other goats (No. 521 and 740), except goat No. 52, may have shown similar changes. Eventually, parotid duct ligation caused alteration in the pathway of phosphorus output; that is,

it induced changes in the comparative amount of phosphorus in blood plasma and that in the alimentary tract. Phosphorus release from bone had been expected to change, but it is unknown in this experiment. The results of this experiment agree with those reported by Mayer et al. [14], who observed in cows that the ratio of urinary to fecal phosphorus output had been changed, but that the phosphorus balance had not been changed by injection with parathyroid extracts. Judging from the marked increase of plasma inorganic phosphorus in this experiment, however, the effect of parotid duct ligation on phosphorus homeostasis differs from that of the action of parathyroid hormone. Scott [20] reported that when sheep and calves were fed all concentrate diets, their urine was acidic and contained a larger amount of phosphorus than when they were fed roughage diets, but that there was no difference in the parathyroid hormone level of blood between the two types of diets.

The rumen epithelium is almost impermeable against phosphorus [6]. Only a small amount of phosphorus enters the digestive tract below the level of the pylorus endogenously [1]. Therefore, main factors which affect the fecal phosphorus output are the following two: phosphorus excretion via saliva and phosphorus absorption from the intestine. It seems that the decrease in fecal phosphorus output after duct ligation may be due to the decrease in phosphorus inflow via parotid saliva endogeneously.

Parotid duct ligation brought about a decrease in ruminal inorganic phosphorus level to 29–47% of the value determined in the control period. This decrease is due to the absence of inflow of a large quantity of phosphorus into the rumen via parotid saliva. Then the inorganic phosphorus

level tended to return progressively to the initial one. The salivary glands other than the parotid also excrete a large amount of phosphorus [11, 30]. Consequently, it is presumed that they may secrete phosphorus in compensation. Tomas et al. [24, 25] emphasized that salivary phosphorus was the major principal determinant of inorganic phosphorus level in rumen fluid. The results of this experiment lend support to their emphasis.

Urinary phosphorus excretion was parallel with titratable acid excretion. Increased phosphorus after parotid duct ligation is eliminated with hydrogen ion as acid phosphate (H_2PO_4^-). This substance causes a decrease in urine pH and acts as a titratable acid.

There was a large individual difference in urinary phosphorus excretion (Table 3). This difference was noticed in sheep [19, 31] and cattle [13, 14]. Meyer [15] found sheep which had excreted 50–60% and less than 1% of the total phosphorus output, respectively, in urine. He reported that sheep which had excreted a large amount of phosphorus in urine showed a high plasma inorganic phosphorus level. In man, phosphaturia owing to genetic deficiency in some enzymes of the renal tubular cells was reported to be expressed as vitamin-D resistant rickets in children and idiopathic osteomalacia (Milkman's syndrome) in adults [8]. In the ruminant, genetic abnormality of urinary phosphorus excretion has not been reported as yet, although it seems to exist.

Parotid duct ligation caused a decrease in urinary sodium excretion temporarily in urine analysis. It is assumed that this phenomenon may probably be induced by the change of extracellular fluid distribution. Parotid duct ligation, however, caused no changes in plasma sodium level,

and urinary sodium excretion returned to the level determined in the control period within two weeks after ligation. Therefore, it is considered that sodium homeostasis may be maintained at the same level as determined in the control period in the 3rd and 4th weeks after ligation. It was expected that parotid duct ligation might cause a decrease in sodium recycling via the salivary glands and an increase in urinary sodium excretion. Contrary to this expectation, it seemed that the sodium excretion from the kidney might be controlled efficiently by the adrenal cortex or the pituitary posterior lobe, and parotid duct ligation might have no effect on sodium homeostasis. The parotid gland and the kidney are important target organs of aldosterone and are closely related to sodium metabolism. The parotid gland releases a large amount of sodium and concerns the osmotic regulation of ruminal fluid. The kidney controls sodium homeostasis readily in response to the sodium status of the animal.

The decrease in ruminal sodium level after parotid duct ligation is partly due to the stopping of sodium inflow via parotid saliva. At that time, sodium level decreased to 68–84% of that determined in the control period. This decrease, however, was lower in degree than that in ruminal inorganic phosphorus level. Sodium is absorbed actively through the rumen epithelium [6]. Therefore, it is not disregarded that sodium transfer in the rumen epithelium is influenced by the osmotic change of ruminal digesta, as shown by Stacy et al. [21]. Ruminal sodium level tended to return progressively to the initial level. It seems that this may be attributed partly to the compensatory secretion by some other salivary glands other than the parotid.

A slight increase in urinary potassium excretion for the first two days after ligation seems to be due to the tissue damage caused by surgery. It is assumed that the mild increase in ruminal potassium level after ligation may be due to the compensatory change for the decrease in sodium level.

Eventually, the ligation of the parotid duct caused an increase in urinary phosphorus excretion and plasma phosphorus level, as shown by Tomas et al. [26]. In addition, it induced a great decrease in ruminal phosphorus level. These results indicate the importance of parotid salivation in phosphorus homeostasis. The duct ligation gave rise to the urinary titratable acidity, but caused little change in the blood acid-base balance. On the other hand, the sodium and potassium contents of urine, feces and blood plasma hardly underwent any change. Therefore, it seems that the homeostasis of sodium and potassium may scarcely be influenced by the ligation of the parotid duct.

The outline of the present paper was printed in the Proceedings of the 77th Meeting of the Japanese Society of Veterinary Science in April, 1974.

Acknowledgments: The author wishes to thank Dr. Shoichiro Omori, of his Institute, for valuable advice.

References

- [1] Annenkov, B. N., Madison, V. L., and Fomichev, Ju. P. (1970). K voprosu o metabolizme fosfora v organizme ovec. *Sel'skhoz. Biol.*, **5**, 405-410. Abstracted in *Nutr. Abst. Rev.*, **41**, 119 (1971).
- [2] Bailey, C. B. (1961). Saliva secretion and its relation to feeding in cattle. 4. The relationship between the concentrations of sodium, potassium, chloride and inorganic phosphate in mixed saliva and rumen fluid. *Brit. J. Nutr.*, **15**, 489-498.
- [3] Clark, R. C., Beal, A. M., Budtz-Olsen, O. E., Cross, R. B., French, T. J., Nave, J. M., and O'Callaghan, M. (1968). The effects of intravenous infusion of acid, alkali and phosphate in sheep. *Aust. J. exp. Biol. med. Sci.*, **46**, p. 15.
- [4] Clark, R. C., Budtz-Olsen, O. E., Cross, R. B., Finnermore, P., and Baueri, P. A. (1973). The importance of the salivary glands in the maintenance of phosphorus homeostasis in the sheep. *Aust. J. agric. Res.*, **24**, 913-919.
- [5] Cullen, G. E. (1922). Studies of acidosis. XIX. The colorimetric determination of the hydrogen ion concentration of blood plasma. *J. biol. Chem.*, **52**, 501-515.
- [6] Dobson, A. (1961). In *Digestive Physiology and Nutrition of the Ruminant*, Lewis, D., editor, Butterworths, London, 69-70.
- [7] Fiske, C. H., and Subbarow, Y. (1925). The colorimetric determination of phosphorus. *J. biol. Chem.*, **66**, 375-400.
- [8] Harper, H. A. (1969). In *Review of Physiological Chemistry*, 12th ed., Lange Medical Publications, Los Altos, California, 404 and 429. Japanese translation by Miura, Y. (1971), Maruzen, Tokyo, 384 and 407.
- [9] Henderson, L. J., and Palmer, W. W. (1914). On the several factors of acid excretion. *J. biol. Chem.*, **17**, 305-315.
- [10] Hungate, R. E. (1966). In *The Rumen and Its Microbes*. Academic Press, New York, 163-170.
- [11] Kay, R. N. B. (1960). The rate of flow and composition of various salivary secretion in sheep and calves. *J. Physiol. (Lond.)*, **150**, 515-537.
- [12] McDougall, E. I. (1948). Studies on ruminant saliva. 1. The composition and output of sheep's saliva. *Biochem. J.*, **43**, 99-109.
- [13] Manston, R., and Vagg, M. J. (1970). Urinary phosphate excretion in the dairy cow. *J. agric. Sci. (Camb.)*, **74**, 161-167.
- [14] Mayer, G. P., Ramberg, C. F., Jr., and Kronfeld, D. S. (1968). Parathyroid influences upon phosphorus balance and homeostasis in cows. *J. Nutr.*, **95**, 202-206.
- [15] Meyer, H. (1972). Über eine Anomalie der renalen Phosphor-Ausscheidung bei Schafen. *Dtsch. tierärztl. Wschr.*, **79**, 426-427.
- [16] Morris, S., and Ray, S. C. (1939). The effect of a phosphorus deficiency on the protein and mineral metabolism of sheep. *Biochem. J.*, **33**,

- 1209-1216.
- [17] Reed, W. D. C., Elliott, R. C., and Topps, J. H. (1965). Phosphorus excretion of cattle fed on high-energy diets. *Nature (Lond.)*, **208**, 953-954.
- [18] Schales, O., and Schales, S. S. (1941). A simple and accurate method for the determination of chloride in biological fluid. *J. biol. Chem.*, **140**, 879-884.
- [19] Scott, D. (1969). Renal excretion of potassium and acid by sheep. *Quart. J. exp. Physiol.*, **54**, 412-422.
- [20] Scott, D. (1972). Excretion of phosphorus and acid in the urine of sheep and calves fed either roughage or concentrate diets. *Quart. J. exp. Physiol.*, **57**, 379-392.
- [21] Stacy, B. D., and Warner, A. C. I. (1966). Balances of water and sodium in the rumen during feeding: Osmotic stimulation of sodium absorption in the sheep. *Quart. J. exp. Physiol.*, **51**, 79-93.
- [22] Tillman, A. D., and Brethour, J. R. (1958). Dicalcium phosphate and phosphoric acid as phosphorus sources for beef cattle. *J. Anim. Sci.*, **17**, 100-103.
- [23] Tillman, A. D., Brethour, J. R., and Hansard, S. L. (1959). Comparative procedures for measuring the phosphorus requirement of cattle. *J. Anim. Sci.*, **18**, 249-255.
- [24] Tomas, F. M., Moir, R. J., and Somers, M. (1967). Phosphorus turnover in sheep. *Aust. J. agric. Res.*, **18**, 635-645.
- [25] Tomas, F. M. (1973). Parotid salivary secretion in sheep: Its measurement and influence on phosphorus in rumen fluid. *Quart. J. exp. Physiol.*, **58**, 131-138.
- [26] Tomas, F. M., and Somers, M. (1974). Phosphorus homeostasis in sheep. I. Effect of ligation of parotid salivary ducts. *Aust. J. agric. Res.*, **25**, 475-483.
- [27] Tomas, F. M. (1974). Phosphorus homeostasis in sheep. II. Influence of diet on the pathway of excretion of phosphorus. *Aust. J. agric. Res.*, **25**, 485-493.
- [28] Tomas, F. M. (1974). Phosphorus homeostasis in sheep. III. Relationship between the amount of salivary phosphorus secreted and the quantities of phosphorus excreted via the urine and faeces. *Aust. J. agric. Res.*, **25**, 495-507.
- [29] Topps, J. H., Reed, W. D. C., and Elliott, R. C. (1966). Studies of the metabolism of cattle given high concentrate diets. *J. agric. Sci. (Camb.)*, **66**, 233-240.
- [30] Tribe, D. E., and Peel, L. (1963). Total salivation in grazing lambs. *Aust. J. agric. Res.*, **14**, 330-339.
- [31] Young, V. R., Lofgreen, G. P., and Luick, J. R. (1966). The effects of phosphorus depletion and of calcium and phosphorus intake on the endogenous excretion of these elements by sheep. *Brit. J. Nutr.*, **20**, 795-805.