Reprod. Nutr. Dévelop., 1985, **25** (2), 389-397.

Intestinal absorption and secretion of total and lipid phosphorus in adult sheep fed chopped meadow hay

A. THÉWIS, E. FRANÇOIS (*)

Faculté des Sciences Agronomiques de l'Etat, Chaire de Productions animales, 5800 Gembloux, Belgium.

(*) *Centre de Recherches Agronomiques de l'Etat, Station de Chimie et de Physique agricoles, 5800 Gembloux, Belgium.*

Summary. The flow of total (P_T) and lipid (P_{PL}) phosphorus was measured in adult sheep fed meadow hay and fitted with Ivan-Johnston reentrant cannulas in the duodenum (just posterior to the entry of the common bile and pancreatic duct) and in the terminal ileum. The pattern of plasma, duodenal and ileal P_T and P_{PL} specific radioactivity was also studied.

The considerable total and lipid P secretion observed between the mouth and the duodenal cannula could be accounted for by salivary and biliary secretions, respectively. This secretion was followed by high absorption in the small intestine and less in the large intestine.

A comparison of P specific radioactivities showed that the selective P_{PL} reabsorption occurring in the small intestine could be due to the existence of an entero-hepatic cycle of biliary phospholipids.

Introduction.

Ruminant utilization of minerals can be studied by a wide range of methods. When phosphorus (P) is investigated, these methods namely include regression and radioisotopic procedures for estimating endogenous loss and true availability, and *in vitro* and *in vivo* techniques for identifying gastrointestinal sites of mineral absorption and secretion. These techniques are often complementary.

Many workers have localized the gastrointestinal sites of P absorption and secretion by using non-absorbable markers in slaughter experiments with sheep (Poppi and Ternouth, 1979 ; Théwis, François and Thielemans, 1978). Others have measured the flow of P along the digestive tract of sheep fitted with intestinal cannulas (Bruce *et al.*, 1966 ; Pfeffer, Thompson and Armstrong, 1970 ; Grace, Ulyatt and MacRae, 1974 ; Leibholz, 1974 ; Ben-Ghedalia *et al.*, 1975).

On the other hand, several scientists have measured true P absorption while estimating endogenous P faecal excretion by the isotopic dilution method (Kleiber *et al.*, 1951 ; Guéguen, 1962 ; Compère, 1966 ; Field, Munro and Suttle, 1977 ; Field *et al.*, 1982 ; Braithwaite, 1981). This method is based on the assumption

that P secreted into the intestinal content has the same specific radioactivity as the P of the blood plasma.

In a slaughter experiment using ^{144}Ce as an unabsorbable marker, we observed considerable total and lipid P secretion in the duodenum of sheep, followed by absorption of the same order of magnitude in the jejunum and ileum (Théwis, François and Thielemans, 1978). Moreover, the specific radioactivity of P in duodenal digesta was sometimes higher than that observed for plasma P (François, 1974). If gastrointestinal P secretions have a higher specific radioactivity than observed for plasma, their magnitude and the efficiency of endogenous P absorption could affect the validity of results obtained by the isotopic dilution method.

In our previous paper, the amounts of P secreted in the duodenum were higher than those reported in the literature and therefore questionable ; moreover, slaughter experiments have several drawbacks (Miller, 1972). In the present paper, the flow of total (P_T) and lipid (P_{PL}) P was measured in adult sheep fed meadow hay and fitted with Ivan-Johnston reentrant cannulas in the duodenum and the terminal ileum. The patterns of plasma, duodenal and ileal P_T and P_{PL} specific radioactivity were also investigated.

Material and methods.

Animals, diet and housing. — About 3 weeks before the experiments began, four Texel rams about 15 months old and weighing between 38 and 49 kg were fitted with reentrant Ivan-Johnston (1981) cannulas in the duodenum (just posterior to the entry of the common bile and pancreatic duct) and in the terminal ileum approximately 20 cm anterior to the ileocaecal junction.

The sheep received 907 $\pm$ 33 g DM/day of chopped meadow hay (particle size : max. 5 cm) and were fed hourly from an automatic device. Crude protein content was 10.9 % (DM basis) and DM digestibility was 0.53. Daily P intake amounted to 2.16 $\pm$ 0.28 g/sheep. The animals were adapted to the diet for at least 30 days before the experiment began.

^{144} Ce was used as an unabsorbable marker to correct the total 24-h flow of digesta through the reentrant cannulas. During the experiment, the hay was labelled with the radiomarker by spraying a solution of ^{144}Ce uniformly on the feed. After drying, we mixed the bulk of food required for each animal throughout the experiment. Further details are given elsewhere (François and Théwis, 1976). This process allowed strong adsorption of the ^{144}Ce on the hay particles, regular administration of the marker and uniform labelling of the digesta.

The animals were housed in metabolism cages with continuous lighting and had free access to water.

Experiment 1. — Five days before collection started, three sheep received hay labelled with radiocerium ; the fourth sheep was used as a digesta donor.

The experimental period included 4 days of total faecal collection followed by 48-h ileal collection and, about 2 days later, by 48-h duodenal collection.

The digesta were collected into Erlenmeyer flasks immersed in ice-water. Every 15 min, an amount of digesta equivalent to that collected, coming either from the donor sheep or from previous collections, was warmed to 39 °C and gradually returned to the sheep. Every 2 or 3 h, the digesta collected were weighed and mixed, and a 10 % sample was taken for analysis. To obtain sufficient dry matter for subsequent analysis, aliquots from the duodenum were pooled over 8-h periods and those from the ileum over 6-h periods.

Experiment 2. — The same animals, diet and housing as in experiment 1 were used, except that the hay was not labelled. Four mCi of ^{32}P as Na_2HPO_4, dissolved in 2 ml of neutral solution, were injected subcutaneously into sheep 1 and 2, while sheep 3 received 12 mCi of ^{32}P.

On days 13, 14 and 15, intestinal digesta were collected for 1 1/2 h first from the duodenal and then from the ileal cannula. A subsample (10 %) was collected at about 15-min intervals and the remainder was gradually returned to the intestine. The aliquots were pooled daily. Just before ileal collection started, blood was taken from the jugular vein using iodoacetate-sodium heparinate as an anti-coagulant. The plasma was separated by centrifugation and a trichloroacetic acid (TCA) filtrate of plasma was prepared immediately after collection. The samples were stored at − 20 °C.

Analysis. — All food, digesta and faeces samples were freeze-dried and then finely ground (1-mm sieve : Clyclotec mill). The lipids were quantitatively extracted from these samples by successively soaking them first in warm solvents (ethanol, chloroform and chloroform-methanol mixture ; 2 : 1 V/V) and then in a cold chloroform-methanol nitric acid mixture (645 : 323 : 33 V/V/V). Lipid P was estimated from the P contents of the lipid extracts, (Théwis, François and Thielemans, 1978). The samples of food, faeces, digesta, urine and their respective phospholipid extracts, as well as blood TCA filtrates, were wet-ashed with a nitro-perchloric acid mixture (2 : 1 V/V). When necessary, the organic solvents were removed previously by evaporation.

P was determined colorimetrically in the solutions according to the method of Misson (1908) using a Technicon autoanalyser. ^{32}P was measured with a gas flow counter after evaporation of a known amount of the solution in a plastic dish.

For ^{144}Ce counting, 0.5 g of dry food, digesta or faeces was thoroughly mixed with 4 g of non-radioactive ground faeces, 0.5 g of casein and a few drops of water. This mixture was shaped into hard discs using a laboratory press. The discs (about 8 mm thick) were counted with a gas flow counter.

Paired t-tests were used for statistical analysis.

Results.

Recovery of ^{144}Ce in digesta and faeces and flow of intestinal contents through the cannulas. — The recovery of ^{144}Ce in the digesta and faeces is shown in table 1 ; the mean daily recovery of the marker in the faeces largely exceeded 100 %. This might be due to the short collection period and/or to the occasional failure of the feeding system during the preexperimental period.

TABLE 1

Recovery of ^{144}Ce and variations in its concentration in the duodenal and ileal digesta and the faeces of sheep receiving a chopped hay diet ; 24-h flows of dry matter at the collection sites.

Collection site	Collection period	Recovery of ^{144}Ce (% of intake)		Coefficient of variation of ^{144}Ce concentrations (%)		Corrected dry matter flow (g/day) (1)		Calculated dry matter flow (g/day) (2)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Proximal duodenum	0-24 h	85.1	2.68	2.7	0.40	558	9.5	560	6.5
	25-48 h	99.8	4.17						
Terminal ileum	0-24 h	85.0	0.55	3.4	0.46	407	7.0	408	4.3
	25-48 h	94.6	0.48						
Faeces	0-96 h	121.5	1.96			342	12.4		

(1) Corrected 24-h flow = corrected for 100 % recovery of ^{144}Ce.

(2) Calculated 24-h flow = $\frac{\text{daily marker intake (CPM)}}{\text{marker concentration during 8- or 6-h collection periods (CPM/g)}}$

Table 1 also shows higher recovery of ^{144}Ce at the cannulas during the second 24-h period of collection.

Another important aspect of this experiment was the low variation in ^{144}Ce concentration in the duodenal and ileal digesta throughout the 6 or 8-h collection period, leading to calculated dry matter flows that were very close to the corrected values (table 1).

Flow of total (P_T) and lipid (P_{PL}) P along the digestive tract and net absorption and net secretion from and into various regions (table 2). — While virtually the same amounts of P_T were excreted and ingested, the quantities of P_T and P_{PL} at the duodenal cannula were significantly higher ($P < 0.01$) than those in the diet and those leaving the ileum. This implies considerable net secretion of P_T and P_{PL} between the mouth and the junction of the common bile duct and duodenum, followed by net absorption from the small intestine. Although P_T and P_{PL} faecal values were lower than the amounts leaving the ileum, the differences were significant only for P_{PL}.

TABLE 2

Flow and net absorption (1) of total (P_T) and lipid (P_{PL}) P along the digestive tract of sheep receiving a chopped hay diet.

Item	P_T		P_{PL}	
	Mean	SEM	Mean	SEM
Amount, mg/24 h				
In diet	2 161	28.3	84	9.1
Entering duodenum	7 280	270.3	384	7.6
Leaving ileum	2 935	60.2	123	10.0
In faeces	2 197	161.6	62	6.1
Net absorption (1), %				
Stomachs and proximal duodenum	− 237	9.0	− 367	53.6
Small intestine	201	7.7	320	48.0
Large intestine	34	6.7	73	6.4
Whole gastro-intestinal tract	− 2	6.2	26	1.1

(1) Net absorption $= \frac{\text{absorbed}}{\text{ingested}} \times 100$

Specific radioactivity of plasma P (SRP_{TCA}) and total (SRP_T) and lipid (SRP_{PL}) P at the duodenal and ileal cannulas. — In this experiment, duodenal and ileal collection was delayed by 2 h ; this roughly corresponded to the transit time of the digesta through the small intestine of the sheep. As shown in table 3, the SRP_T was not different at the duodenal and ileal cannulas. Moreover, in all cases, SRP_T at the duodenal site was lower than SRP_{TCA}. On the other hand, SRP_{PL} decreased from the duodenum to the ileum, and SRP_{PL} was very close to that of the plasma and sometimes higher.

TABLE 3

Comparison of specific radioactivity of plasma inorganic P (SRP_{TCA}), total P (SRP_T) and lipid P (SRP_{PL}) in the duodenal and ileal digesta of 3 sheep during 3 consecutive days after ^{32}P injection (mean ± SEM).

Day	SRP_{TCA}	SRP_T		Significance of the difference	SRP_{PL}		Significance of the difference
		Duodenum	Ileum		Duodenum	Ileum	
1	1	0.87 ± 0.04	0.86 ± 0.06	N.S.	0.97 ± 0.01	0.71 ± 0.03	**
2 ([1])	1	0.87 ± 0.03	0.86 ± 0.04	N.S.	0.98 ± 0.06	0.85 ± 0.09	N.S.
3	1	0.86 ± 0.02	0.88 ± 0.03	N.S.	1.05 ± 0.03	0.82 ± 0.04	**

N.S. : not significant ; ** : $P < 0.01$.
([1]) Only two sheep.

Discussion.

The Ivan-Johnston cannula (Ivan and Johnston, 1981) presents several advantages as compared with the Ash reentrant cannula (Ash, 1962) : (1) it does not involve transection of the intestine and (2) it provides representative samples of digesta without using a dual-phase marker. Moreover, it is easy to insert the cannulas, maintain the external part, and collect and reintroduce the digesta. Post-surgical recovery was rapid and the cannula was seldom blocked. Unfortunately, in our experiment, there was generally some leakage at the ileal cannula of the sheep about 3 months after surgery, and we could not keep the animals in good condition for more than 3 1/2-4 months. Leakage appeared later at the duodenal cannula. At slaughter, we observed that the intestine had grown through the arterial prosthesis of woven dacron and was free of it. This was also reported by Poncet *et al.* (1982).

In spite of the fact that we used a regular feeding design and administered ^{144}Ce uniformly, faecal marker recoveries higher than 100 % were observed (table 1). In previous experiments we had often noticed the irregular faecal excretion of the marker, especially when the animals were stressed or when the faeces were collected over a too-short period of 3 or 4 days. Generally, 100 % recovery was only achieved over a 10-day period. Since in this experiment neither the marker nor its administration were responsible for the irregular secretion, we adjusted the flow for 100 % recovery of ^{144}Ce.

On the other hand, the duodenal flow ranged from 13 to 63 g DM/2 h, while the ileal flow varied from 26 to 57 g DM/3 h. When the amounts of digesta collected were pooled over longer periods (6 h at the duodenal site and 8 h at the ileal site), the flow was more regular and variations in marker concentration in the digesta were rather low (table 1). Therefore, the flow of digesta could be calculated with good accuracy over 6 (duodenum) or 8 (ileum)-h periods (table 1).

As far as P movement across the gut wall is concerned, we observed considerable net secretion between the mouth and the duodenal cannula. This agrees with the results of Pfeffer *et al.* (1970), Grace *et al.* (1974), Ben-Ghedalia

et al. (1975), Théwis *et al.* (1978), Poppi and Ternouth (1979) and Ben-Ghedalia, Tagari and Geva (1982). The large amounts of saliva secreted continuously by the sheep and possible abomasal secretion explain this observation. From the results of Kay (1960), it may be calculated that if no P absorption occurs before the small intestine, 3 to 8 g of P enter the rumen each day via the saliva. Moreover, in our experiment, P from biliary phospholipids also contributed to the amount of P entering the duodenal cannula. Adams and Heath (1963) calculated that 10-15 g of phospholipid or 0.4-0.6 g of P_{PL} enter the sheep gut every day in the bile. The contribution of undegraded and ruminal endogenous phospholipids (Kurilov and Firsov, 1974) was not determined in this experiment.

The net absorption of P_T in the small intestine reported in our experiment has also been mentioned by several other authors (Pfeffer *et al.*, 1970 ; Ben-Ghedalia *et al.*, 1975, 1982 ; Poppi and Ternouth, 1979 ; Grace *et al.*, 1974 ; Théwis *et al.*, 1978). Table 2 shows that P_{PL} is largely absorbed in the small intestine. Unfortunately there is a lack of quantitative information on this subject in the literature. Lennox *et al.* (1968) reported the progressive disappearance of phospholipids through the intestine of sheep. Hydrolysed biliary phospholipids, together with bile salts, would help the micellar solubilization of fatty acids in sheep intestine and enhance the lipid absorption (Leat and Harrison, 1969, 1974 ; Harrison and Leat, 1972 ; Lough and Smith, 1976).

In our experiment, the amounts of P_T and P_{PL} secreted in the proximal duodenum were not as high as observed in a previous experiment with slaughtered sheep (Théwis *et al.*, 1978) ; in the latter trial, they were probably due to excessive secretion of mineral and biliary organic P at slaughter or to the sloughing-off of the epithelium during emptying.

Selective intestinal absorption of biliary phospholipids is also confirmed by the decrease of SRP_{PL} from the duodenal to the ileal content. All these observations would suggest the existence of an entero-hepatic cycle of biliary phospholipids in sheep intestine similar to that found by Boucrot (1972) in rats.

The fact that SRP_{PL} at the duodenal site was sometimes higher than SRP_{TCA} may be explained by the biliary secretion. Indeed, specific radioactivity in the bile of sheep slaughtered 13 days after labelling with ^{32}P was higher than the value found in the plasma TCA filtrate (3.47 *vs* 2.78, respectively) (François, 1974). However, this observation does not alter the validity of the isotope dilution method of Kleiber *et al.* (1951), owing to the intestinal reabsorption of biliary phospholipids.

Reçu en janvier 1984.
Accepté en novembre 1984.

Acknowledgements. — This study was supported by grant n° 2.4521.79 from « Fonds de la Recherche Fondamentale Collective. »

Résumé. *Absorption et sécrétion intestinales du phosphore total et phospholipidique chez le mouton adulte nourri au foin de prairie haché.*

Le flux de P total (P_T) et de P lipidique (P_{PL}) est étudié chez 3 béliers adultes nourris au foin de prairie et munis de canules réentrantes au niveau du duodénum post-cholédoque et de l'iléon terminal. Chez ces mêmes animaux, on compare l'évolution des activités

spécifiques du P plasmatique à celles du P_T et du P_{PL} au niveau duodénal et iléal. Les résultats montrent une sécrétion du P_T et P_{PL} entre la bouche et le duodénum. Si la première traduit un apport important de P d'origine salivaire, la seconde doit essentiellement être attribuée à la sécrétion de phospholipides biliaires.

Cette sécrétion est suivie d'une absorption intense de P_T et de P_{PL} dans l'intestin grêle, voire même dans le gros intestin. L'évolution comparée des activités spécifiques de P plasmatique et de celles de P_T et de P_{PL} au niveau du duodénum et de l'iléon traduit une absorption sélective du P lipidique ce qui pourrait s'expliquer par l'existence d'un cycle entero-hépatique des phospholipides biliaires chez le mouton.

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