# Phosophorus kinetics in the sheep

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1. Ten castrated young adult sheep were brought indoors and given lucerne chaff (four animals with a phosphorus intake of 2·40 g/d) and fresh Ruanui ryegrass (six animals with a P intake of 3·72 g/d) to determine various kinetic indices of their P metabolism.

2. The results obtained from combined nutritional balance and $^{32}P$ radioactivity measurements were analysed using the simulation, analysis and modelling computer (SAAM) program. Since the plasma P specific activity curve was described by at least four exponential terms, a four compartmental model ($M_1$, $M_2$, $M_3$ and $M_4$) was used to describe the experimental values.

3. The mean mass of the total exchangeable P pool ($M_T$) for the lucerne chaff-fed and Ruanui ryegrass-fed sheep was 25·5 and 28·8 g respectively, while the total P transport ($V_T$) to the exchangeable pool ($M_T$) was markedly lower for sheep given lucerne chaff compared to those given Ruanui ryegrass (54·1 *v.* 111·3 mg/kg body-weight per d). Likewise the rate of P absorption ($V_a$), faecal endogenous loss of P ($V_f$), urinary P excretion ($V_u$) as well as the movement of P to ($V_o^+$) and from ($V_o^-$) the skeleton and soft tissue was also lower in the lucerne chaff-fed sheep.

4. The availability of P, that is the proportion of ingested P which is absorbed, was 0·56 and 0·62 for the sheep given chaff and fresh herbage respectively.

5. The P metabolism of one sheep (sheep no. 30) differed from the other sheep in that its plasma inorganic P concentration was higher (95 *v.* 54 mg/l). This was associated with a larger first compartment ($M_1$), a more rapid P transport between $M_1$ and $M_2$, an increase in ($V_a$) and a marked increase in ($V_u$).

In contrast to calcium (Ramberg *et al.* 1970) little is known concerning the size of the exchangeable phosphorus pool of the body or the rates of P inflow and outflow to and from this pool in the ruminant. Further, recent studies (Playne, 1976; Sykes & Dingwall, 1976) have suggested that the P requirement of sheep as well as P availability from various diets including pasture and pasture hay should be re-evaluated. Quantitative information on many indices of P metabolism such as the losses of P in the urine, the faecal endogenous loss of P and the quantities of P absorbed are needed.

This investigation describes the application of modelling techniques using results collected from balance studies and $^{32}P$ isotope measurements to study P kinetics in the young adult sheep. A preliminary report has been presented (Grace, 1979).

## EXPERIMENTAL

### *Sheep*

Castrated Romney Marsh sheep aged 18–24 months, weighing 38–40 kg were housed indoors in metabolism crates designed for isotope studies and the handling of faeces and urine.

### *Diets*

All sheep had been grazing pasture containing (g/kg) 3–4 P and 5–6 Ca before being brought inside and given 850 g dry matter (DM) as lucerne (*Medicago sativa*) chaff (Expt 1, four animals) and later 'Grasslands Ruanui' perennial ryegrass (*Lolium perenne* L.) (Expt 2, six sheep). The Ruanui ryegrass was cut in the spring at a height of 100–150 mm daily at 08.00 hours and subsampled and DM determined by the procedures of Ulyatt & MacRae (1974).

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Both feeds were offered at hourly intervals from automatic feeders. The mean daily P intakes for the sheep given lucerne chaff and Ruanui ryegrass were 2·40 and 3·72 g respectively while the associated mean daily Ca intakes were 12·0 and 4·5 g respectively. Distilled water was freely available.

### *Experimental design and collection of samples*

After a 28 d pre-experimental training period each animal was given, as a single dose, via an indwelling cannula placed in the right jugular vein, 100 $\mu$Ci $^{32}P$ in 20 ml sterile isotonic saline (9 g/l sodium chloride). Blood samples (10 ml) were taken by vacutainer from the left jugular vein at accurately-timed intervals approximately 1, 2·5, 5, 10, 20, 40, 60 and 80 min after the administration of $^{32}P$. Further samples were obtained at 2, 4, 6, 9, 12, 18, 24, 27, 30 h and then daily at 09.00 hours for the next 14 d. The blood was centrifuged, 5 ml plasma removed and 6 ml trichloroacetic (100 g/l; TCA) added before the contents were recentrifuged to give a clear TCA supernatant fraction containing the plasma inorganic P. The daily outputs of faeces and urine were measured, and subsamples stored at $-10°$. Faeces were dried at 105° for 48 h for DM determination.

### *Analytical*

Dried ground samples of diet and urine and faeces were wet-ashed (nitric acid–sulphuric acid 4:1, v/v) and decolourized with hydrogen peroxide (300 g/l) in the terminal stages of the ashing process. The plasma TCA supernatant fraction and wet-ash material from the faeces and urine were then transferred to counting vials and made up to a total volume of 16 ml. Suitable standards were prepared from a subsample of the $^{32}P$ solution which was administered to the sheep. The radioactivity was measured using Cerenkov radiation in a Searle Analytic Isocap-300 counter.

P was determined by the method of Murphy & Riley (1962) and the Ca by atomic absorption spectrophotometry.

### *P model*

The simulation, analysis and modelling (SAAM) computer program was used to fit a model to the experimental values (Fig. 1). The use, assumptions and limitations of the model have been discussed (Berman *et al.* 1962; Berman, 1965). Calculations of the compartmental P masses and rates of P transport involve the assumption of a steady-state during the experiment. The notation used for the P model is similar to the Ca model described by Aubert *et al.* (1963) and Ramberg *et al.* (1970). Total exchangeable P pool is designated as $M_T$ and $M_1$, $M_2$, $M_3$ and $M_4$ are the four compartments within the pool. P transport ($V_T$) is the total flow of P into the pool ($M_T$) and in a steady-state situation is equal to the outflow. The outflow of P from $M_T$ consists of the urinary P ($V_u$), the faecal endogenous loss of P ($V_f$) and the P deposition into non-exchangeable bone and uptake by soft tissues ($V_o^+$). The total P inflow to $M_T$ is the sum of the P absorbed from the digestive tract ($V_a$) and the P removed from the bone and soft tissues $V_o^-$. $V_a$ is calculated as the difference between the P intake ($V_I$) and faecal P output ($V_F$) after correcting for the faecal endogenous P loss ($V_a = V_I-(V_F-V_f)$). The efficiency of absorption ($\alpha$) is the proportion of the P ingested which is absorbed ($\alpha = V_a/V_I$).

$\lambda_{21}$, is the fractional rate of transport of P from compartment $M_1$ to compartment $M_2$. $R$ is the rate of transport and more specifically $R_{21} = M_1\,\lambda_{21}$ which is the rate of stable P transported from $M_1$ to $M_2$. The fractional rates of the unidirectional transport of P into urine, faeces and bone are given by the symbols $\lambda_u$, $\lambda_f$ and $\lambda_o^+$ respectively.

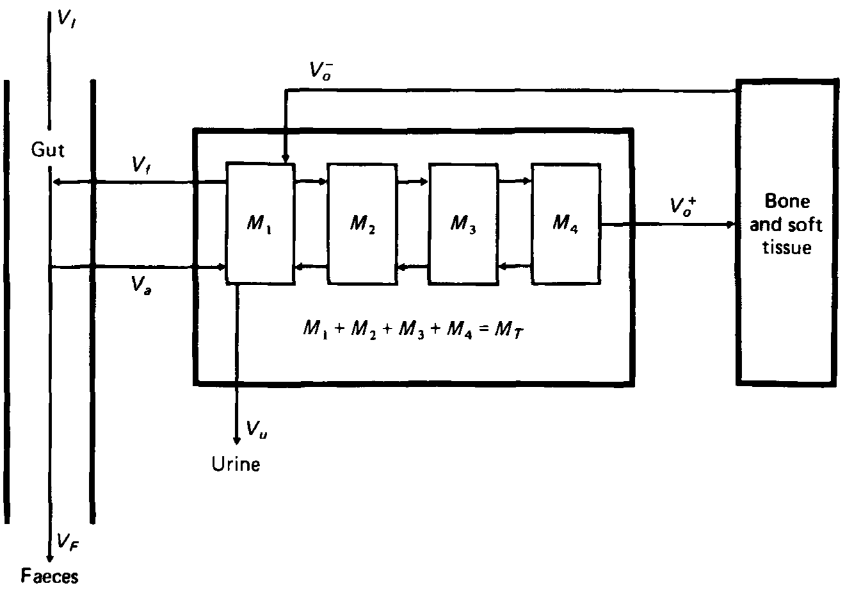


Fig. 1. Scheme of phosphorus metabolism in the sheep. $M_1$, $M_2$, $M_3$ and $M_4$ are compartments of the exchangeable P pool $M_T$. $M_1$ is the site of tracer administration and is sampled via the blood. Inflows of P from the gut ($V_a$) and the bone together with the soft tissues ($V_o^-$) enter $M_1$ while the excretory losses of P to the faeces ($V_f$) and urine ($V_u$) leave $M_1$. $M_4$ is the site of P loss to the non-exchangeable bone and soft tissues.

## RESULTS

### *Plasma inorganic P specific activity and cumulative excretion of* $^{32}P$

Mean values (four sheep given lucerne chaff and five sheep given Ruanui perennial ryegrass: values for sheep no. 30 are given separately) for the plasma inorganic P levels and changes in the plasma inorganic P specific activity with time are shown in Table 1 and Fig. 2 respectively.

The accumulative excretion of $^{32}P$ in the faeces and urine are presented in Fig. 3. A four compartment model was used to describe the experimental values (Fig. 1) because it was found that at least four exponential terms were required to fit the entire curve of the plasma P specific activities. Again values for sheep no. 30 from the Ruanui ryegrass-fed group are presented separately because its P metabolism differed markedly from the rest of the group. For all sheep, except sheep no. 30, most of the $^{32}P$ was excreted via the faeces with only small amounts being lost in the urine. Further, the faecal $^{32}P$ losses were, in the latter part of the study, greater for the sheep given lucerne chaff when compared to the animals given Ruanui ryegrass. In sheep no. 30 the quantities of $^{32}P$ excreted via faeces were less than for the other sheep given the Ruanui ryegrass but this was compensated for by the fact that considerably more of the $^{32}P$ was excreted via the urine.

### *Total exchangeable P pool* ($M_T$)

The mean $M_T$ values were observed to be similar, namely 25·4 and 28·8 g for the lucerne chaff-fed and Ruanui ryegrass-fed sheep respectively. From the compartment masses ($M$), the rate of P transport between the compartments ($R_{21}$, $R_{12}$, $R_{32}$, $R_{23}$, $R_{43}$ and $R_{34}$) and the P outflows ($V_f$, $V_u$ and $V_o^-$) the mean turnover rates of the four compartments can be calculated (Fig. 1 and Table 1). The mean turnover times for $M_1$, $M_2$, $M_3$ and $M_4$ in sheep

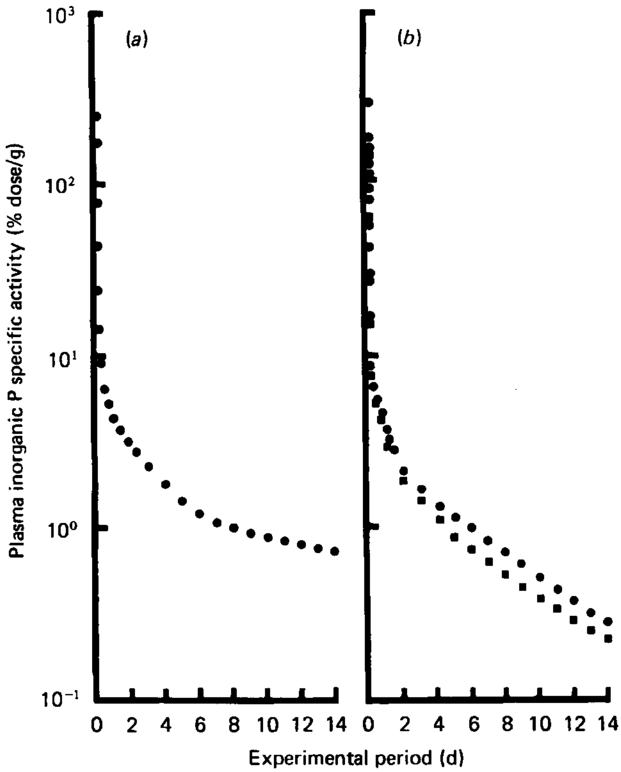


Fig. 2. Mean plasma phosphorus specific activities (% dose/g) of sheep given (*a*) lucerne chaff (●) and (*b*) Ruanui ryegrass (●) with the values of sheep no. 30 (■) presented separately.

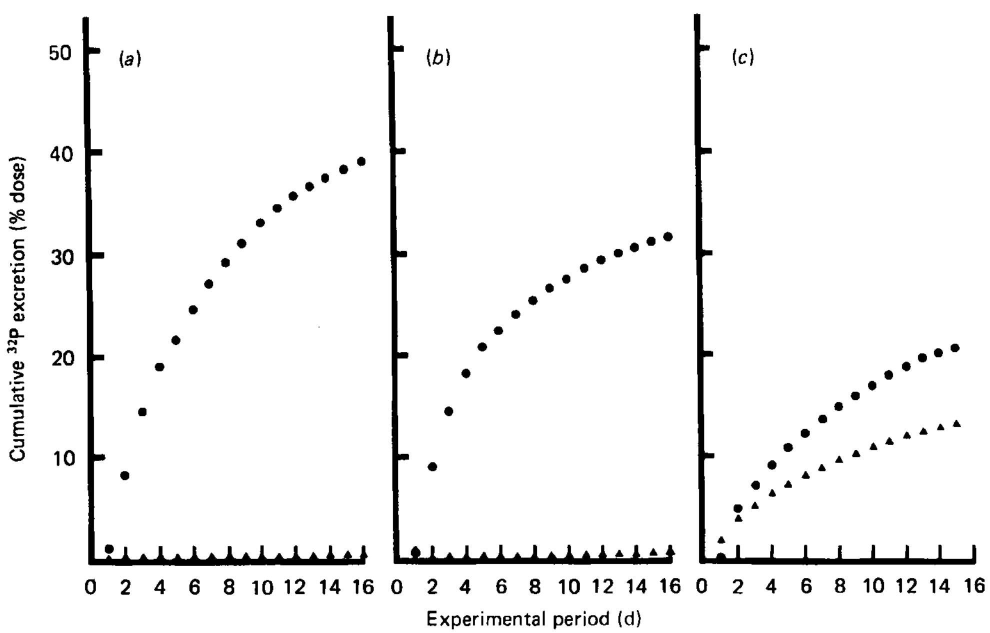


Fig. 3. The mean cumulative $^{32}$P excretion (% dose; faeces (●) and urine (▲) in sheep given (*a*) lucerne chaff (*b*) Ruanui ryegrass and (*c*) sheep no. 30 given Ruanui ryegrass.

Table 1. *Compartment masses (g), rates of phosphorus transport (mg/kg body weight per d) and plasma inorganic P levels (mg/l) in sheep given lucerne chaff (P intake 2·40 g/d) and Ruanui perennial ryegrass (P intake 3·72 g/d)*

| | | Lucerne chaff | | | | Ruanui perennial ryegrass | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sheep no. ... | | 15 | 10 | 36 | 148 | 3 | 5 | 17 | 83 | 138 | 30 |
| Mass of P in compartment 1 | $M_1$ | 0·122 | 0·139 | 0·096 | 0·124 | 0·342 | 0·340 | 0·306 | 0·309 | 0·246 | 0·518 |
| Mass of P in compartment 2 | $M_2$ | 0·876 | 1·240 | 1·660 | 0·638 | 1·090 | 1·340 | 0·928 | 0·799 | 0·840 | 1·010 |
| Mass of P in compartment 3 | $M_3$ | 8·50 | 11·20 | 11·91 | 7·58 | 9·75 | 10·10 | 8·61 | 9·37 | 9·87 | 10·90 |
| Mass of P in compartment 4 | $M_4$ | 11·8 | 19·9 | 14·5 | 11·6 | 17·69 | 19·30 | 15·20 | 16·50 | 19·60 | 17·80 |
| Mass of total exchangeable P pool | $M_T$ | 21·298 | 32·479 | 28·166 | 19·94 | 28·872 | 31·080 | 25·044 | 26·978 | 30·556 | 30·228 |
| Transport of P from $M_1$ to $M_2$ | $R_{21}$ | 464 | 650 | 415 | 244 | 1038 | 1137 | 1271 | 1587 | 1240 | 2100 |
| Transport of P from $M_2$ to $M_1$ | $R_{12}$ | 438 | 622 | 398 | 218 | 974 | 1067 | 1205 | 1516 | 1167 | 2033 |
| Transport of P from $M_2$ to $M_3$ | $R_{32}$ | 295 | 425 | 368 | 236 | 472 | 715 | 652 | 637 | 677 | 625 |
| Transport of P from $M_3$ to $M_2$ | $R_{23}$ | 269 | 408 | 350 | 208 | 407 | 645 | 584 | 567 | 605 | 559 |
| Transport of P from $M_3$ to $M_4$ | $R_{43}$ | 134 | 81 | 80 | 60 | 161 | 247 | 241 | 242 | 194 | 191 |
| Transport of P from $M_4$ to $M_3$ | $R_{34}$ | 108 | 52 | 635 | 33 | 97 | 178 | 173 | 171 | 121 | 126 |
| Rate of P intake | $V_I$ | 61·5 | 60·0 | 60·0 | 61·5 | 95·3 | 93·0 | 97·8 | 97·8 | 93·0 | 95·4 |
| Rate of loss of P in faeces | $V_F$ | 55·3 | 55·0 | 58·0 | 52·8 | 73·8 | 74·7 | 85·8 | 77·1 | 72·0 | 54·1 |
| Rate of excretion of P in urine | $V_u$ | 0·33 | 0·34 | 0·20 | 0·20 | 0·62 | 0·46 | 0·18 | 0·24 | 0·40 | 22·6 |
| Endogenous faecal P | $V_f$ | 29·7 | 33·3 | 27·7 | 23·1 | 33·3 | 47·5 | 41·0 | 41·3 | 38·0 | 32·8 |
| Availability of dietary P | $\alpha$ | 0·58 | 0·63 | 0·50 | 0·52 | 0·58 | 0·70 | 0·54 | 0·63 | 0·63 | 0·77 |
| Rate of P absorption | $V_A$ | 35·9 | 38·2 | 29·8 | 31·8 | 54·8 | 65·7 | 53·1 | 62·1 | 59·0 | 74·1 |
| Total P inflow (and outflow) into $M_T$ | $V_T$ | 56·1 | 64·7 | 45·2 | 50·2 | 97·7 | 117·0 | 108·9 | 112·1 | 111·5 | 120·7 |
| Rate of movement of P into bone and tissues | $V_0^+$ | 26·1 | 28·0 | 17·2 | 26·9 | 63·8 | 69·2 | 67·9 | 70·5 | 73·2 | 65·1 |
| Rate of movement of P from bone and tisues | $V_0^-$ | 20·2 | 16·5 | 15·5 | 18·5 | 42·8 | 51·2 | 55·8 | 50·0 | 52·5 | 46·6 |
| P balance | $\Delta$ | 8·5 | 11·5 | 1·7 | 8·4 | 21·0 | 18·0 | 12·1 | 20·5 | 20·7 | 18·5 |
| Plasma inorganic P | | 48 | 54 | 54 | 44 | 55 | 53 | 57 | 67 | 52 | 95 |

given lucerne chaff were estimated to be 9·36 min, 53·5 min, 15·06 h and 4·14 d respectively while the comparable values for the sheep given Ruanui ryegrass were 8·92 min, 18·9 min, 7·76 h and 2·13 d respectively.

### *P inflow* ($V_a$ *and* $V_o^-$) *and P outflow* ($V_f$, $V_u$ *and* $V_o^+$)

The total transport of P ($V_T$) into or out of the exchangeable P pool was lower (54·1 *v.* 111·3 mg/kg body-weight per d) in the lucerne chaff-fed sheep compared with those fed Ruanui ryegrass (Table 1). Likewise the absorption of P from the digestive tract ($V_a$), the movement of P from the bone and soft tissues ($V_o^-$) as well as the secretion of P back into the digestive tract ($V_f$) and the movement of P to the bone and soft tissues ($V_o^+$) were all lower in the lucerne chaff-fed shep compared to the Ruanui ryegrass-fed sheep.

### *Availability of dietary P* ($\alpha$)

There was considerable individual sheep variation in the availability (the absorbed P expressed as a fraction of the P intake) of dietary P. However the mean P availability for the sheep given lucerne chaff was a little lower than for the sheep given Ruanui ryegrass (0·56 *v.* 0·62).

### *Sheep no.* 30

Quantitatively many of the indices of P metabolism measured in sheep no. 30 were different from the rest of the sheep given Ruanui ryegrass (Table 1). While the mass of its exchangeable P pool ($M_T$) was similar to many of the other sheep its compartmental mass of $M_1$ and P transport ($R_{21}$ and $R_{12}$) between $M_1$ and $M_2$ was nearly twice that of other sheep. Further, the daily amounts of P absorbed ($V_a$) and excreted via the urine ($V_u$) in sheep no. 30 were markedly greater than for other sheep while the faecal endogenous loss of P ($V_f$) tended to be lower.

However the movement of P to and from the bone and soft tissues ($V_o^+$ and $V_o^-$) as well as the retention of P within the body was found to be similar for all sheep.

## DISCUSSION

### *The P model*

The scheme for the P model outlined in Fig. 1 was developed using the experimental values collected from the nutritional balance studies and the $^{32}P$ kinetic experiments. The P exchangeable pool ($M_T$) is represented by four interchanging compartments ($M_1$–$M_4$) which reflect a dilution of the $^{32}P$ in progressively larger masses of P. The compartments cannot be defined in terms of anatomical structures but are more likely to be related to metabolic P processes having similar turnover rates. Further it seems most likely that compartment $M_1$ includes the plasma inorganic P and the P in rapid equilibrium with it while compartment $M_4$, because of its size and relatively slow turnover rate, would include some skeletal P.

### *Exchangeable P pool* ($M_T$) *and P transport* ($V_T$)

An important assumption made in making up the model is that a steady state is maintained which means that the mass of P within each compartment should remain constant and the inflow of P into the exchangeable P pool should equal the P outflow. The strict routine of feeding at hourly intervals was assumed to have minimized diurnal variation in the secretion of salivary P into the rumen in an attempt to achieve the necessary steady state conditions.

The mass of the exchangeable P pool, $M_T$, (20–30 g) is approximately 10–12% of the total body P and the range of values for each group of sheep was similar regardless of the diet or P intake. In the sheep given Ruanui ryegrass where the P intake was higher than that of those given lucerne chaff, it was found that the inflow and outflow ($V_T$) of P to and from the exchangeable P pool, the transport rate of P between compartments, the rates of movement of P to and from bone and soft tissue as well as the quantities of P absorbed and secreted were markedly increased. This reflects the dynamic nature of P metabolism in the sheep and shows that the amount of P ingested can alter the rates of P transport including its absorption from the secretion into the digestive tract. However, since the daily Ca intake of the lucerne chaff-fed sheep was 12·0 g (Ca:P, 5) while that of the Ruanui ryegrass-fed sheep was 4·5 g (Ca:P, 1·2) there is the possibility that the high intake of Ca could influence the P metabolism of the lucerne chaff-fed sheep. High Ca intakes cause a decrease in the secretion of parathyroid hormone and the synthesis of 1,25 dihydroxycholecalciferol (Arnaud, 1978) and as a result the quantities of P absorbed, mobilized and excreted could be decreased (Wadsworth & Cohen, 1976). In the absence of any information on plasma parathyroid hormone and 1,25 dihydroxycholecalciferol levels and since the plasma Ca concentrations (95–105 mg/l) were similar for all sheep while the P intakes from both diets were adequate to the P requirements of the sheep it does not seem likely, in this study, that the high Ca intakes of the lucerne chaff-fed sheep would have greatly influenced their P metabolism.

The possibility that the faecal endogenous loss of P appears to be related to the P intake has some important implications when estimating P requirements because the faecal endogenous loss and urinary excretion of P are important components in the model used to determine the P requirements of sheep. Further investigations are required to define more accurately the relationship between P intake and the faecal endogenous loss of P before new estimates of P requirements can be determined.

### *Availability of P* ($\alpha$)

The availability of P ($\alpha$) reflects a number of complex processes and is the difference between the amount of P absorbed from and that secreted into the digestive tract expressed as a fraction of the P intake. Further the faecal endogenous loss ($V_f$) is a measure of the net secretion of P into the digestive tract because although large quantities of P (two to three times the P intake) are secreted into the rumen via the saliva considerable amounts of this P together with the dietary P are absorbed from the small intestine. Likewise the P absorption factor ($V_a$) does not reflect the total quantity of P that is transported across the wall of the digestive tract. The total amount of P absorbed is equal to the total secretion of P into the lumen of the gut plus the P excreted in the urine and the P retained by the body.

In this study a wide variation in the availability of P was observed on both diets while mean values for the lucerne chaff-fed and Ruanui ryegrass-fed sheep were 0·56 and 0·62 respectively. These values are lower than those reported for many diets consisting of mixtures of dried sugar beet pulp, cottonseed pulp, maize extract, lucerne meal, cassava (*Manihot esculenta* Crantz) and various phosphates (Playne, 1976). Up to now nothing was known about P availability from fresh pasture or pasture hay except for lucerne hay (Playne, 1976) and therefore, it is suggested from the findings of this study that, under pastoral farming conditions it would be more realistic to consider the P availability for the grazing sheep as being near 0·60 rather than higher values of 0·80 which are more applicable to other diets.

### *Sheep variation in phosphorus metabolism*

An unexpected, but interesting finding was that the P metabolism of sheep no. 30 was markedly different from other sheep in this study. Its plasma P level was the highest (95 *v.* 54 mg/l) and this was associated with an increase in the rate of P absorbed, while the mass of $M_1$ and transport rates $R_{21}$ and $R_{12}$ were greater than for other sheep. Further, sheep no. 30's handling of the absorbed P differed in that both the urine ($V_u$) and the faecal endogenous loss ($V_f$) were important routes of outflow from the exchangeable P pool whereas in most sheep only small amounts of P are excreted via the urine. A high rate of urinary excretion of P has also been reported in a number of sheep fed various diets (Skyes & Dingwall, 1976; Field *et al.* 1977), and as the P intake was increased it was found that proportionally more of the P lost from the body was excreted in the urine rather than returned to the digestive tract via the saliva. Furthermore, as demonstrated by sheep no. 30 when compared with others in this study, there were large differences between individual sheep in the way in which they partition the amounts of P loss via the urine and saliva. Under conditions of low P intakes, which are encountered by the grazing sheep, the secretion of the P to the digestive tract for reabsorption is a successful way of conserving P which would otherwise be lost to the animal if excreted in the urine.

The mechanism(s) which determine the quantities of absorbed P which are excreted in the urine or secreted in the saliva is unknown but sheep which are urinary excretors appear to have high plasma inorganic P levels. Since parathyroid hormone has been shown to increase the salivary secretion of P (Tomas, 1974) as well as its urinary excretion in sheep (Barlet & Care, 1972) it is speculated that another, as yet unidentified, hormonal factor could be important in mediating a reciprocal relationship between the urinary and salivary P.

Variations in the mineral metabolism of sheep have been reported in other studies, for example differences in the sodium and potassium concentrations of red blood cells (Evans, 1954) and in their ability to absorb copper (Suttle, 1974).

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