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# Renal Response to Intravenous Phosphate Infusion in the Sheep

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*Abstract*

Renal clearance experiments were performed on six Merino ewes in which plasma phosphate concentrations were increased by the intravenous infusion of isohydric sodium phosphate. As the phosphate load to the kidney increased, the renal tubular reabsorptive capacity became saturated and a definite tubular maximum for phosphate reabsorption ($Tm_P$) was demonstrated. The $Tm_P$ was directly related to the glomerular filtration rate and had a mean value of $333 \cdot 1 \pm 27 \cdot 0$ (S.E.M.) $\mu$mol/min or $416 \cdot 6 \pm 13 \cdot 5$ $\mu$mol/100 ml glomerular filtrate. Calcium infused concurrently with phosphate in order to maintain plasma total calcium levels did not alter the $Tm_P$. Ultrafilterability of calcium and phosphate in the plasma decreased with phosphate infusion and this was accentuated by an accompanying calcium infusion.

The $Tm_P$ in sheep's kidney is higher than in non-ruminant animals and the implications of this are discussed.

## Introduction

In ruminants, particularly sheep, only about 1% or less of the total phosphorus excretion usually occurs via the urine when the animals eat herbaceous diets (see, for example, Otto 1932; Stacy 1969; Scott 1970). There is a high threshold for urinary phosphorus excretion in terms of plasma phosphate levels in these animals (Watson 1933; Hendricks and Seekles 1966) and the kidneys of sheep and cattle appear to be capable of a high rate of phosphate reabsorption (Mayer *et al.* 1966; Scott 1972; Symonds and Manston 1974) relative to that found in non-ruminant animals (Mudge *et al.* 1973; Pitts 1974). However, none of the information available conclusively demonstrates the existence of a maximal capacity for phosphate reabsorption by the renal tubules ($Tm_P$) in ruminant animals. The present paper describes experiments which clearly show the presence of a $Tm_P$ in the sheep's kidney and that this $Tm_P$ is high.

## Materials and Methods

### *Animals*

Mature Merino ewes were used. They received daily a maintenance diet consisting of 500 g wheaten hay chaff and 500 g lucerne hay chaff which provided 55–65 mmol phosphorus and 250–330 mmol calcium. Each animal was denied food on the day it was used in an experiment but water was always available *ad libitum*. An indwelling polyethylene cannula was placed into each jugular vein of the sheep on the day before an experiment and filled with heparinized saline.

*Experimental Protocol*

In each infusion experiment the procedures detailed below were performed in conjunction with standard renal clearance techniques (Smith 1956). After the insertion of a Foley self-retaining catheter into the bladder and the removal of a pre-infusion blood sample, a priming dose of 5% inulin (Fluka) at 0·5 ml/kg body wt was given followed by a continuous infusion of 2% inulin at 1 ml/min. About 30 min was allowed for equilibration and then collections for two baseline (control) clearance periods were made. All clearance periods were of 20 min duration and when urine flow rates were less than about 1·5 ml/min the bladder was rinsed with warm distilled water at the end of each period.

**Table 1. Results of an experiment with one animal (9149) in which both calcium and phosphate were infused**

UF plasma, ultrafilterable plasma

| Total concurrent time (min) | Urine flow (ml/min) | Glomerular filtration rate (ml/min) | Phosphate | | | | |
|---|---|---|---|---|---|---|---|
| | | | UF plasma concn (mM) | Filtered through glomeruli (μmol/min) | Excreted in urine (μmol/min) | Reabsorbed by tubules (μmol/min) | Ratio reabsorbed/filtered |
| 0 | Infuse with 2% inulin, 1 ml/min; prime with 5% inulin, 0·5 ml/kg body wt | | | | | | |
| 30– 50 | 9·60 | 81·4 | 1·31 | 106·7 | 0·00 | 106·7 | 1·000 |
| 50– 70 | 8·05 | 76·9 | 1·15 | 88·4 | 0·008 | 88·3 | 0·999 |
| 70 | Infuse with 2% inulin, 1 ml/min; 0·32M phosphate, 3 ml/min; 45 mM $CaCl_2$, 1 ml/min | | | | | | |
| 70– 90 | 3·40 | 76·5 | 2·03 | 151·2 | 0·07 | 151·1 | 0·999 |
| 90–110 | 2·53 | 86·4 | 3·44 | 297·2 | 11·6 | 285·6 | 0·961 |
| 110–130 | 2·30 | 87·6 | 4·41 | 386·1 | 81·3 | 304·8 | 0·789 |
| 130–150 | 2·58 | 89·8 | 4·97 | 446·5 | 159·2 | 287·4 | 0·644 |
| 150–170 | 2·80 | 91·6 | 5·57 | 510·2 | 214·7 | 295·5 | 0·579 |
| 170–190 | 2·48 | 95·6 | 6·14 | 587·0 | 285·2 | 301·8 | 0·514 |
| 190–210 | 2·45 | 94·2 | 6·60 | 621·6 | 302·2 | 319·5 | 0·514 |
| 210 | Infuse with 2% inulin, 1 ml/min; cease phosphate and calcium infusions | | | | | | |
| 210–230 | 2·68 | 97·9 | 6·19 | 606·0 | 292·1 | 313·9 | 0·518 |
| 230–250 | 4·25 | 88·3 | 5·28 | 466·3 | 174·2 | 292·0 | 0·626 |
| 250–280 | 2·79 | 83·8 | 4·60 | 385·6 | 117·2 | 268·4 | 0·696 |
| 280–300 | 2·20 | 88·3 | 3·90 | 344·3 | 77·0 | 267·3 | 0·776 |

Immediately following the control clearance collections, a continuous intravenous infusion of either phosphate or phosphate plus calcium was begun and continued for 140 min (seven periods). In all experiments an isohydric sodium phosphate solution of 0·32–0·36M phosphate was infused at about 3 ml/min [24 μmol phosphate/(min kg body wt)]. In the five experiments where calcium was infused concurrently with phosphate, a solution of calcium chloride was infused at 1 ml/min via a separate indwelling cannula located caudally to the blood sampling cannula in the contralateral jugular vein. The infusion provided 55 μmol calcium/(min kg body wt). Clearance collections were continued for a further 80 min after cessation of the phosphate and calcium infusions.

Blood samples for ultrafiltration were taken immediately after the routine clearance blood samples in periods 1, 2 (control), 3, 5, 7, 9, 11 and 13. They were withdrawn anaerobically into a heparinized syringe in a manner similar to that described by Radde *et al.* (1971) and centrifuged within the hour. After centrifugation, the plasma was transferred anaerobically into another syringe. The plasma pH remained constant for up to 16 h but ultrafiltration was always performed within about 4 h of sample collection.

*Analytical Procedures*

Plasma, plasma ultrafiltrate and urine samples were analysed for phosphate and inulin by auto-analysis, for calcium by atomic absorption spectroscopy and for sodium by flame photometry using methods outlined previously (Tomas *et al.* 1973). Ultrafiltration of plasma samples was done essentially by the method described by Stafford and Edwards (1973). The filtration rate was increased by surrounding the dialysis tubing with nylon gauze. The pH of plasma immediately prior to ultrafiltration was between 7·35 and 7·40 which is within the normal range.

## Results

### *Renal Phosphate Reabsorption*

The detailed protocol and results of a typical infusion experiment in one animal (No. 9149) which received concurrent calcium and phosphate infusions are presented in Table 1. The amount of phosphate reabsorbed by the renal tubules (TRP) appears to reach a plateau as the amount of phosphate filtered through the glomeruli (GFP) increases. An example of the relationship between the TRP and the GFP is given in Fig. 1 which shows the data obtained from four infusions in one sheep (No. 6037) and clearly demonstrates the presence of a $Tm_P$. In this case the data are calculated from total inorganic phosphate levels in the plasma since this is consistent with comparable studies reported in the literature. Fig. 1 also illustrates both the apparent decline in the efficiency of phosphate reabsorption which often occurred in the last three periods after cessation of the phosphate infusion (values shown below curved dashed line), and the finding that this decline was always prevented by the concurrent infusion of calcium.

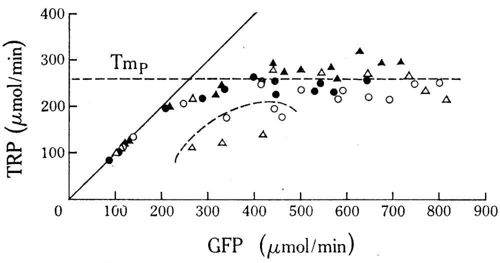


**Fig. 1.** Relationship between the amount of phosphate filtered by the glomeruli (GFP) and that reabsorbed by the renal tubules (TRP) in four separate experiments on one sheep (6037). Closed symbols are data from experiments with concurrent calcium infusion. Points below curved dashed line are the last three collection periods of each experiment without calcium infusion (open symbols).

A summary of the $Tm_P$ data from all experiments and related information on the calcium status of the animals is given in Table 2. The infusion of calcium appears to have little, if any, effect on the $Tm_P$ but the use of ultrafilterable phosphate data considerably reduces the mean $Tm_P$ obtained (see below).

The changes in plasma total and ultrafilterable calcium and inorganic phosphate concentrations are shown in Fig. 2. Plasma inorganic phosphate levels were increased by about 10 mM above control values by the infusion. The ultrafilterable phosphate levels in the control plasma samples were 96% of the plasma inorganic phosphate levels (range 92–102%) but these values fell by about 5% during phosphate infusion alone, and by 10–15% when calcium accompanied the phosphate infusion. Total calcium levels in the plasma declined about 40% during the phosphate infusions but the ultrafilterable component was affected to a greater extent and was reduced from 60 to less than 50% of the total. Although a concurrent calcium infusion increased the total calcium concentrations, the ultrafilterable levels declined similarly to those without calcium infusion, and consequently fell from 60 to less than 30% of the total. A comparison between TRP data drawn either from total or ultrafilterable plasma phosphate values obtained from three infusions on one animal is shown in Fig. 3. The plateau of TRP, which indicates the $Tm_P$, is more distinct when the ultrafilterable phosphate data is used and the average $Tm_P$ is reduced by 20%. The mean $Tm_P$ values obtained using the ultrafilterable phosphate data (see Table 2) show a reduction in the $Tm_P$ of about 15% for experiments with phosphate infusion alone and about 25% where calcium also is infused compared with values obtained using total inorganic phosphate levels. If the ultrafilterability of phosphate in all samples taken during maximal TRP is assumed to be 90% in experiments where this was not measured, then the average $Tm_P$ is $328 \cdot 6 \pm 15 \cdot 6$ $\mu$mol/100 ml glomerular filtrate. This is 21% lower than the mean value determined using total inorganic phosphate values in plasma.

**Table 2. Summary of mean data from all infusions**
GF, glomerular filtrate; n.d., not determined

| Sheep No. | Infused Ca | Mean $Tm_P$ | | | Peak plasma total Ca (mM) | Max. change in UF Ca in plasma (mM) |
|---|---|---|---|---|---|---|
| | | ($\mu$mol/min) | ($\mu$mol/ 100 ml GF) | ($\mu$mol/ 100 ml GF) (UF)[A] | | |
| 7229 | − | 360 | 441 | | | n.d. |
| 6271 | − | 212 | 364 | | | n.d. |
| | − | 143 | 282 | | | n.d. |
| 6037 | − | 234 | 383 | | | n.d. |
| | − | 272 | 436 | | | n.d. |
| | + | 246 | 403 | | 3·42 | n.d. |
| | + | 288 | 433 | 334 | 3·22 | −1·30 |
| 8266 | − | 466 | 428 | | | n.d. |
| | + | 401 | 413 | | 2·66 | −1·25 |
| 9149 | − | 472 | 481 | 404 | | −1·60 |
| | − | 361 | 455 | 394 | | −1·35 |
| | + | 402 | 432 | 325 | 2·65 | −1·10 |
| 9212 | − | 428 | 476 | 408 | | −1·13 |
| | + | 379 | 405 | 256 | 3·68 | −1·05 |
| Mean | | 333·1 | 416·6 | 353·5 | | |
| S.E.M. | | 27·0 | 13·5 | 24·4 | | |

[A] Calculation of GFP based on ultrafilterable (UF) phosphate values.

### *Glomerular Filtration Rate (GFR) and the $Tm_P$*

The data from all sheep show the mean $Tm_P$ found in an experiment to be directly related to the corresponding mean GFR. This is shown in Fig. 4 which also includes a plot showing the distribution of the $Tm_P$/GFR ratio for all animals. Only three $Tm_P$/GFR values are less than 4 mM and these were obtained from animals which showed significant urinary phosphorus excretion during the control period (fractional excretion $\geqslant 0 \cdot 01$).

There was no consistent relationship between $Tm_P$/GFR and the control pre-infusion plasma phosphate level.

### *Excretion of Cations*

The ultrafilterable calcium clearances were not correlated with either sodium or phosphate clearances in the experiments where ultrafilterable levels of calcium in plasma were determined. During the course of an experiment there was a rise in calcium clearance from $<0 \cdot 1$ to *c.* $0 \cdot 4$ ml/min which preceded an increase in sodium clearance. Also, calcium clearances reached a peak and began to fall rapidly some

40–60 min before maximal sodium clearances were attained. A similar pattern for calcium clearance was exhibited in relation to phosphate clearances. That this should be so can be seen in Fig. 5 which shows a positive relationship between the sodium and phosphate clearances in these experiments.

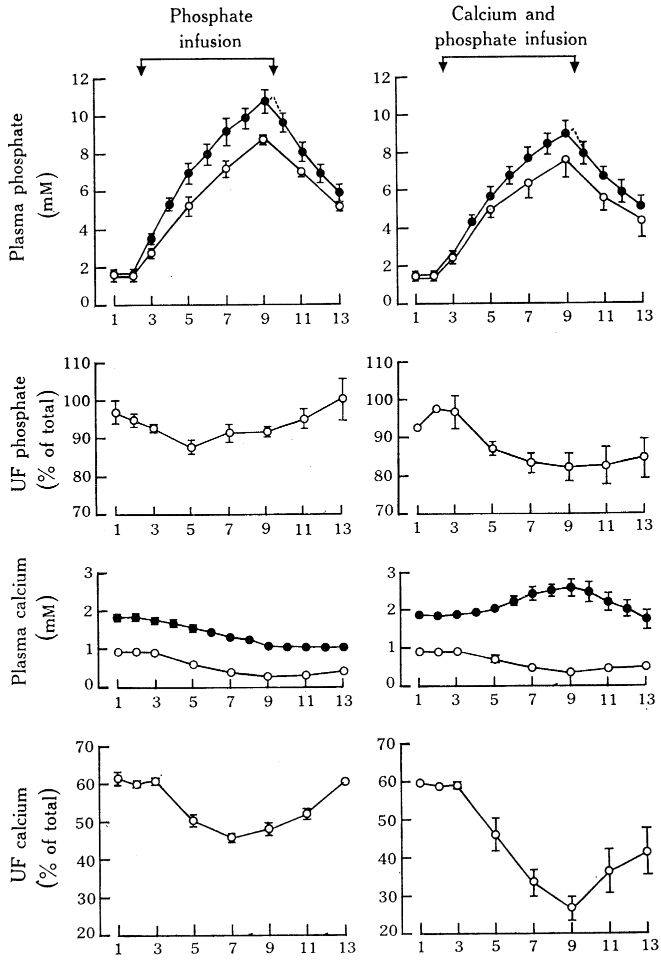


**Fig. 2.** Changes in plasma total (●) and ultrafilterable (○) calcium and inorganic phosphate in eight experiments without and five experiments with calcium infusion. Ultrafilterable (UF) data are from three of the experiments in each case. Vertical bars show standard error of the mean unless this is less than the height of the symbol.

## Discussion

The experiments reported here clearly show that in sheep the renal tubules exhibit a maximal capacity for phosphate reabsorption, at least under the experimental conditions necessary to ascertain this. The $Tm_P$ appears to be much higher than that reported for other animal species and data from comparable experiments on other animals have been drawn together for comparison in Table 3. The comparative data show that even if the $Tm_P$ values for sheep were decreased 21% to allow for non-ultrafilterable plasma phosphate, the renal reabsorptive capacity for phosphate would remain substantially higher than that for non-ruminant animals where this correction has not been made.

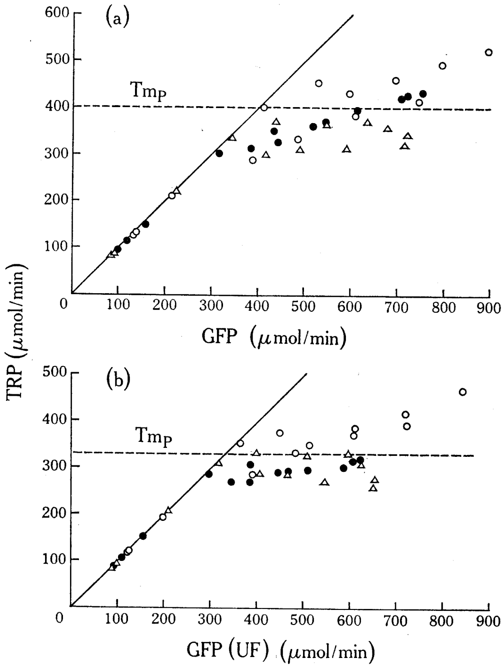


**Fig. 3.** Relationship between the GFP and TRP in three experiments on one sheep (9149). In (*a*) the GFP is calculated from plasma total inorganic phosphate levels and in (*b*) ultrafilterable phosphate levels are used. Symbols and abbreviations are as in Fig. 1.

The $Tm_P$ values reported here may also be overestimated because of the use of continuously increasing plasma phosphate levels to vary the GFP. Any lag in the delivery of urine from the kidney to the bladder would lead to underestimation of excretion relative to the GFP, with the degree of error related to the urine flow rate.

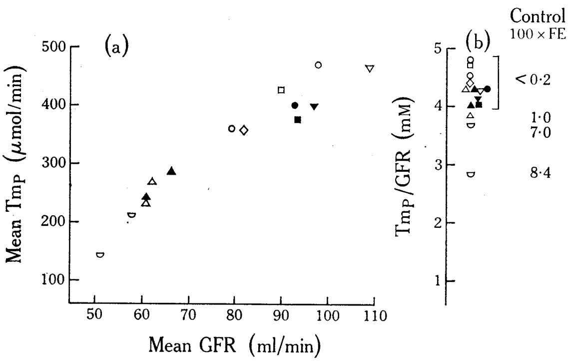


**Fig. 4.** (*a*) Relationship between the mean GFR in each experiment and the corresponding mean $Tm_P$. (*b*) Mean $Tm_P$/GFR ratio for each experiment. Symbols are as in Fig. 1 but distinguish between sheep. Fractional excretion (FE) of phosphate in control periods is shown to the right of the symbols.

However, the GFP itself is underestimated in these experiments, since jugular blood samples have phosphate levels 2–5% lower than arterial samples, due principally to salivary gland activity (Tomas, unpublished data). This would largely cancel errors arising from lags of the order of 2–4 min between urine formation and its collection.

The infusion of calcium concurrently with phosphate did not lead to an increase in the $Tm_P$, suggesting that parathyroid hormone (PTH) secretion was not a factor in these experiments. This is consistent with the data from the phosphate infusion

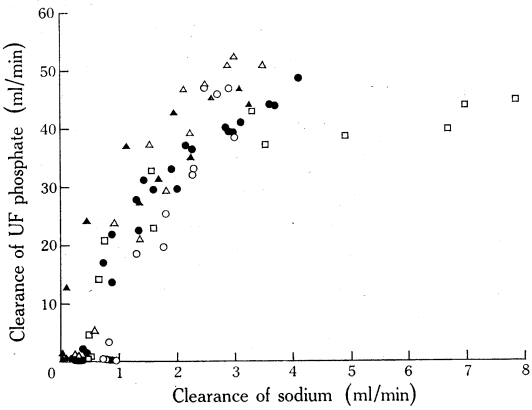


**Fig. 5.** Relationship between the clearance of sodium and the clearance of ultrafilterable (UF) phosphate. Symbols are as in Fig. 1.

experiments in cows reported by Mayer *et al.* (1966). However, although plasma total calcium levels were maintained by the concurrent calcium infusion, the ultrafilterable calcium levels fell regardless to almost the same levels as those when phosphate was

**Table 3. Typical $Tm_P$ values for several animal species**

No correction applied for the non-ultrafilterable fraction of plasma inorganic phosphate. Range of values (where available) given in parentheses

| Animal species | $Tm_P$ | | | Reference |
|---|---|---|---|---|
| | ($\mu$mol/min) | [$\mu$mol/(min kg$^{0\cdot75}$)] | ($\mu$mol/100 ml GF) | |
| Rat | 5·1 (3·2–7·0) | 13·4 (8–18) | 175 (110–220) | Ginn and Shanbour 1967; Frick 1968 |
| Man | 123 (65–200) | 7·7 | 115 (65–160) | Bijvoet 1969 |
| Dog | 110 (70–210) | 10·7 (7–17) | 129 (90–160) | Pitts and Alexander 1944; Foulks 1955; Malvin and Lotspeich 1956; Hellman *et al.* 1964 |
| Cattle | 2160 (1300–3000) | 19·8 (12–27) | 275 (222–316) | Mayer *et al.* 1966; Symonds and Manston 1974 |
| Sheep | 378 (330–450) | 22·6 (19–28) | 490 (415–590) | Tomas 1968 |
| | 720 (680–760) | 36·6 (35–39) | 530 (515–545) | Scott 1972[A] |
| | 333 (143–472) | 19·2 (8–28) | 416 (282–481) | Tomas (this paper) |

[A] Data for sheep on roughage diet. $Tm_P$ not clearly defined. GFRs taken from separate experiment on same sheep.

infused alone. Fischer *et al.* (1973) found PTH secretion increased in cows following phosphate infusion and found this to be related to ionic calcium levels in plasma. On the other hand, Sherwood *et al.* (1968) found that when calcium was infused with phosphate to maintain plasma total calcium, there was no increase in PTH secretion as found with phosphate infusion alone. In the experiments reported here, calcium infusion prevented the fall in $Tm_P$ which usually occurred about 100–120 min from the start of the phosphate infusion (Fig. 1) and this suggests that there may have been an inhibition of PTH secretion but other undefined factors which can increase fractional phosphate excretion independently of PTH may have been involved (Swenson *et al.* 1975). Certainly the diminution of calcium clearances prior to the attainment of the peak sodium or phosphate clearances is consistent with PTH intervention (Agus *et al.* 1973) but this effect also may arise by different mechanisms (Hulley *et al.* 1969).

Although several workers have shown a direct relationship between phosphate and sodium reabsorption by the renal tubule (see Knox *et al.* 1973), the relationship seen here could be expected in that both sodium and phosphate are simultaneously infused. The lack of a relationship between calcium and sodium excretion is probably due either to a direct effect of phosphate or to a possible intervention of PTH or other factors during the later stages of the infusion (Hulley *et al.* 1969).

The plot of the $Tm_P$ against GFR for all experiments (Fig. 4) shows a positive relationship similar to that shown for humans (Bijvoet 1969) and for dogs (Hellman *et al.* 1964). In general, this relationship also holds for data obtained between experiments on the same animals and points to variable glomerular or nephron functioning. However, unlike the case for humans (Bijvoet 1969), there is no relationship between the fasting (control) plasma phosphate levels and the $Tm_P$/GFR ratio. This is not surprising since in the sheep the renal excretion of phosphate is not normally the regulator of plasma phosphate levels (Watson 1933; Tomas 1974) as is the case with man.

It is interesting that the sheep showing the lowest $Tm_P$ values (sheep 271, Table 2) also showed the lowest $Tm_P$/GFR values (Fig. 4) and was the only animal to show substantial excretion of phosphate in the urine during the control periods (approximately 10% of GFP). Bijvoet (1969) found that the $Tm_P$/GFR correlated with parathyroid gland activity and it seems probable that this was the case here since the plasma calcium levels in this sheep were about 0·2 mM lower than in any of the other animals. In one experiment, sheep 037 also showed a lowered $Tm_P$/GFR ($<4$ mM) and here also there was a measurable excretion of phosphate (about 1% of GFP) during the control collection. If these three $Tm_P$ determinations are omitted from the means in Tables 2 and 3, then the mean $Tm_P$ is $370 \pm 23$ $\mu$mol/100 ml glomerular filtrate and $21 \cdot 3 \pm 1 \cdot 2$ $\mu$mol/(min $kg^{0 \cdot 75}$).

The relatively constant $Tm_P$/GFR (numerically equal to the mean plasma threshold for phosphate excretion) indicates that the limits to the phosphate transport system are similar between sheep and that the dependence of total reabsorptive capacity upon GFR may be due to grading of tubular function at varying GFRs (Renkin and Gilmore 1973). It is also possible that the changes in ion and fluid reabsorption rates from within the proximal tubule resulting from the different GFRs may alter the equilibrium tubular fluid to plasma phosphate ratio (Knox *et al.* 1973) and hence the $Tm_P$.

The limitations associated with defining the $Tm_P$ by means of raising the plasma phosphate level have been reviewed and discussed by Mudge *et al.* (1973). Nonetheless, it is obvious from Table 3 that on a comparative basis, ruminant animals have a higher capacity to reabsorb phosphate from the renal tubules than do non-ruminant animals although this difference is more pronounced in sheep than in cattle. The mechanism(s) for this relatively high reabsorption is not yet defined. It seems unlikely that there is any marked difference in the number of nephrons per unit body size of the animals except perhaps a higher number in the rat (Rytand 1938; Renkin and Gilmore 1973). Phosphate reabsorption takes place predominantly in the proximal tubule (Knox *et al.* 1973) and this segment comprises a substantially greater proportion of the renal tubule in herbivores than in carnivores (Sperber 1944). Hence it may be anticipated that the reabsorption of phosphate from the proximal region of the tubule would be relatively greater in herbivores. Alternatively, the reabsorption of phosphate may not be restricted to the proximal tubule in the sheep's kidney.

The kidney does not appear to be a major organ of continuous regulation of plasma phosphate homeostasis in sheep (Tomas 1974) and hence factors which moderate the avidity of phosphate reabsorption (see Knox *et al.* 1973; Beck and Goldberg 1974) may not normally be operative to such an extent in these animals. Such factors or the intracellular phosphate levels or both (Mudge *et al.* 1973) may, however, be important in sheep in mediating the renal phosphate excretion responses to grain feeding (Scott 1972) or restriction of salivary phosphorus secretion (Tomas and Somers 1974). These aspects merit further study in order to gain better understanding of the normally high reabsorptive rate, particularly in relation to the regulation of phosphate excretion in sheep.

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