COMPARATIVE ASPECTS OF GASTROINTESTINAL PHOSPHORUS METABOLISM

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INTRODUCTION

The physiological role of phosphorus as an essential component of many organic and inorganic compounds is well documented. Whereas 80% of total body P is localized within the bone, mainly as hydroxyapatites with varying calcium:P ratio, the remaining 20% in cells, cell membranes and body fluids is a major constituent of different organic compounds and enables the regeneration of energy-supplying substances and contributes as an additional buffer system.

The basic mechanisms of gastrointestinal P metabolism differ substantially when ruminant and non-ruminant species are compared. In ruminants large amounts of inorganic P (P_i) are secreted into the gastrointestinal tract by the salivary glands and this endogenous secretion normally is well balanced by net P_i absorption from the upper small intestines. Neither the transport mechanisms involved nor their regulation is clearly identified. In contrast to single-stomach animals renal P_i excretion is low in ruminants and, thus, does not contribute significantly to the overall regulation of P_i homeostasis. However, individual animals have been reported which are characterized by a significant renal P_i excretion and, thus, behave like single-stomach animals. The reasons for this are unknown.

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It is the aim of the present review to discuss the major differences in gastrointestinal P_i transport mechanisms and their hormonal regulation between ruminant and non-ruminant species. In addition, the effects of dietary P deficiency on microbial metabolism in the rumen are considered.

ENDOGENOUS SECRETION INTO THE GASTROINTESTINAL TRACT

In ruminants the salivary glands are the major site for endogenous P_i secretion into the gastrointestinal tract. The daily secretion rate between 5 and 10 g P_i in sheep and between 30 and 60 g P_i in cows (Breves *et al.* 1987; Reinhardt *et al.* 1988; Scott, 1988) is achieved by both the high salivary flow-rate and the ability of the salivary glands to concentrate P_i in comparison with plasma P_i. However, it has to be considered that the concentration ratio between plasma and saliva from the individual glands may differ substantially. Concentrations between 12·5 and 32 mM were determined in parotid saliva of conscious sheep whereas in the submaxillary glands the P_i concentration ranged between 1 and 5 mM (Kay, 1960). The P_i concentration in parotid saliva is mainly determined by the plasma P_i concentration. Scott & Beastall (1978) and Mañas-Almendros *et al.* (1982) found a linear increase in parotid saliva P_i when the plasma P_i concentrations were increased to 4–5 mM by intravenous P_i infusions. Under these conditions the concentration ratio between saliva and plasma P_i varied between 12:1 and 16:1. Although it could be demonstrated by a micropuncture study that the end-pieces of the parotid gland are the major site for P_i secretion (Compton *et al.* 1980), the transport mechanisms which are involved in this process are still unknown. Recently, Shirazi-Beechey *et al.* (1991) studied the P_i uptake across basolateral membrane (BLM) vesicles (BLMV) obtained from sheep parotid epithelial cells and they postulated the presence of a Na^+-dependent electrogenic P_i transport system. So far, however, no information is available on transport processes across the apical membrane. Controversy still exists about whether P_i secretion is regulated by parathyroid hormone (PTH) or 1,25-dihydroxycholecalciferol ($1,25\text{-}(OH)_2D_3$, calcitriol, vitamin D-hormone; Compton *et al.* 1980; Mañas-Almendros *et al.* 1982; Wright *et al.* 1984).

In single-stomach species salivary P_i concentrations are substantially lower (Nielsen & Petersen, 1970; Coroneo *et al.* 1981; Young & Schneyer, 1981) and, since the daily salivary flow-rates are also lower, salivary P_i secretion only represents a small proportion of total P_i in the intestinal lumen which is available for absorption.

Small amounts of P_i are secreted with gastric and pancreatic juice, bile and intestinal fluid. There is no evidence that these secretions differ significantly between ruminant and non-ruminant species and their overall contribution to gastrointestinal P_i turnover is of minor importance (Wilkinson, 1976; Scott, 1988).

SITES AND MECHANISMS OF GASTROINTESTINAL P_i ABSORPTION

NON-RUMINANTS

Small intestines

It is generally accepted that the small intestines are the major site for P_i absorption in single-stomach species and that this transport is stimulated by vitamin D (Harrison & Harrison, 1961; Wasserman & Taylor, 1976; Radde *et al.* 1980; Jungbluth & Binswanger, 1989). The transepithelial P_i transport consists of both an active saturable and a passive

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non-saturable component (Danisi & Straub, 1980; Hildmann *et al.* 1982; Drüeke & Lacour, 1983; Lee *et al.* 1986*a*).

The active vitamin D-stimulated component which is not coupled to the transcellular Ca transport, depends on the presence of sodium in the lumen compartment (Fuchs & Peterlik, 1980; Peterlik *et al.* 1981; Wasserman, 1988). Controversy still exists as to what extent P_i may be transported paracellularly. However, since the active transport is readily saturable, passive absorption may predominate at high lumen P_i concentrations (Neer, 1979; Reinhardt *et al.* 1988).

Quantitatively P_i absorption differs along the small intestines (Walling, 1977; Danisi & Straub, 1980; Danisi *et al.* 1980; Lee *et al.* 1980). From in vivo studies in rats McHardy & Parsons (1956) concluded that P_i absorption was higher in the jejunum compared with the ileum, and Harrison & Harrison (1961) investigated the effects of vitamin D by applying the everted-sac technique and found the highest transport in the jejunum with decreasing rates of transfer in the duodenum and ileum.

In order to differentiate between passive and active transport processes more recent studies were carried out with porcine and rat small intestines using the in vitro Ussing-chamber technique (Radde *et al.* 1980; Favus, 1985; Jungbluth & Binswanger, 1989). Since these experiments were done in the absence of any chemical or electrical gradients significant transepithelial net fluxes required an active mechanism. In porcine tissues net P_i absorption predominantly occurred in the jejunum and to a lower extent in the duodenum (160 nmol/cm^2 per h *v.* 40 nmol/cm^2 per h). It was negligible in the ileum. Net P_i fluxes obtained from rat small intestines confirmed the jejunum to be the major site for active P_i absorption (Walling, 1977; Jungbluth & Binswanger, 1989). However, the flux-rates were substantially lower in control animals (8 nmol/cm^2 per h) as compared with P-depleted rats (33 nmol/cm^2 per h).

In another series of experiments with small intestines from vitamin D-deficient rats it was shown that in the absence of significant plasma 1,25-$(OH)_2D_3$ levels jejunal net P_i absorption persists at a level of 25 nmol/cm^2 per h and increased in response to 1,25-$(OH)_2D_3$ repletion to about 40 nmol/cm^2 per h (Walling, 1977). Unlike the jejunum, net P_i secretion was present in the duodenum and ileum during vitamin D deficiency. However, 1,25-$(OH)_2D_3$ administration converted the duodenal net P_i secretion to a small but significant absorption and reduced, but did not abolish, net P_i secretion in the ileum. In contrast to the findings with pig and rat small intestines, in rabbit small intestines the active P_i transport was highest in the duodenum. Thus, species differences have also to be considered (Danisi & Straub, 1980). It should be stated, however, that in vitro studies may help to characterize transport mechanisms but they do not allow any conclusive evidence on the major absorptive sites under in vivo conditions.

Despite obvious species differences and varying nutritional and hormonal effects which may modulate small intestinal P_i absorption it may be concluded from the present findings that in non-ruminants the proximal parts of the small intestines are predominant for active P_i absorption in comparison with the distal regions. In addition, it is generally accepted that ions, such as Ca^{2+} or P_i, can only be absorbed in the soluble form. Since the intralumen pH increases along the small intestines the solubility and, thus, the absorption of P_i could be affected by these changes in a similar way as it has been shown for Ca^{2+} in man (Sheikh *et al.* 1990).

Cellular P_i transport consists of at least three steps (Murer & Hildmann, 1981; Karsenty *et al.* 1985*a*; Danisi *et al.* 1988; Shirazi-Beechey *et al.* 1988): (1) P_i entry across the lumen brush-border membrane (BBM) into the enterocyte; (2) intracellular P_i transport from the lumen to the basolateral side of the cell and (3) P_i extrusion across the BLM.

Conclusive evidence for a secondary active Na^+–P_i cotransport system across the BBM

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of rabbit and rat small intestines has been shown by several laboratories, using BBM vesicles (BBMV) (Berner *et al.* 1976; Murer & Hildmann, 1981; Quamme, 1985; Danisi *et al.* 1988; Shirazi-Beechey *et al.* 1988). The maximum velocity (V_{max}) of this symporter is increased by vitamin D-hormone and P deficiency with little or no effect on the Michaelis constant (K_m) (Peterlik & Wasserman, 1978; Quamme, 1985; Caverzasio *et al.* 1987; Danisi *et al.* 1988). It has not yet been clearly elucidated whether the carrier preferentially accepts either the monovalent or the divalent P_i and whether the carrier induces a net charge transfer across the lumen membrane (Cross *et al.* 1990).

Evidence for a Na^+-independent diffusional mechanism for cellular P_i uptake was presented by Danisi & Straub (1980) and Karsenty *et al.* (1985*a*) from studies in rabbits and rats. The molecular structure of the Na^+–P_i symporter still remains to be identified. Possible candidates are the polypeptides with molecular weights between 130 and 155 kD found in different studies with rabbit BBMV (Debiec & Lorenc, 1988; Peerce, 1988, 1989). Using an antibody technique, Shirazi-Beechey *et al.* (1988) have shown that guinea-pig antibodies to rabbit BBMV could inhibit the Na^+-dependent P_i-transport. By this technique another suitable tool might be provided for further identifying and characterizing the lumen P_i carrier protein.

There is only a small amount of information on the mechanisms of intracellular P_i transport. Measurements of intracellular P_i are complicated by metabolic conversion and/or intracellular compartmentalization of P_i. There is some evidence from compartmental analysis of the specific activity of transported P_i that the ion migrates from the lumen to the contralumen pole of the enterocyte in a discrete transport pool which is not readily exchanged with intracellular P_i (Kowarski & Schachter, 1969; Peterlik & Wasserman, 1978).

The intracellular migration of P_i can be blocked by adding cytochalasin B in concentrations which are sufficient to disrupt the microfilament system of absorptive cells (Fuchs & Peterlik, 1979). A second possibility which has received experimental support considers the steroid-like, receptor-mediated action of $1,25\text{-}(OH)_2D_3$, triggering the transcription of specific genes that code for carrier proteins (DeLuca, 1988). The $1,25\text{-}(OH)_2D_3$-stimulated P_i transport can be blocked by inhibitors of RNA and protein synthesis such as actinomycin D, α-amanitin and cycloheximide (Peterlik *et al.* 1981). However, the existence of specific and vitamin D-dependent cytosolic P_i-carrier proteins still has to be proved. Recently, specific phosphate transport proteins were determined in bacterial cells (Luecke & Quiocho, 1990).

The mechanisms of P_i extrusion across the BLM are not yet fully understood. Studies of rat jejunum suggest that it involves a Na^+-independent carrier-mediated mechanism which is driven by the electrochemical gradient (Danisi *et al.* 1984; Quamme, 1985). However, the studies of Ghishan *et al.* (1987) suggest the presence of a high-affinity Na^+-dependent P_i carrier in BLMV prepared from the small intestines of rats. In addition, a similar mechanism has been detected in human small intestinal BLMV (Kikuchi & Ghishan, 1987).

Large intestines

The hind-gut is of minor importance for the overall P_i absorption along the gastrointestinal tract (Cramer, 1972). From studies in pigs no P_i absorption from the large intestines could be detected (Guéguen *et al.*1968; Partridge *et al.* 1986). However, this may be different when pigs are kept on high-P diets (Drochner, 1984; Den Hartog *et al.* 1985). In vitro experiments on colonic P_i transport mechanisms in rats have shown that P_i may be transported by passive diffusion with no significant effects of vitamin D (Lee *et al.* 1980).

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RUMINANTS

Forestomachs and abomasum

Different experimental techniques have been adopted to study the extent to which P_i is absorbed up to the proximal duodenum and which mechanisms are involved. Measurements on concentration differences between rumen fluid and venous plasma or between arterial and venous blood draining the reticulo-rumen did not show any significant phosphate transport (Parthasarathy, 1952; Parthasarathy *et al.* 1952; Yano *et al.* 1978). The permeability of the rumen epithelium for phosphate in both directions could be demonstrated by infusing ^{32}P either intraruminally (Scarisbrick & Ewer, 1951; Parthasarathy *et al.* 1952; Wright, 1955; Pfeffer, 1968) or intravenously (Parthasarathy *et al.* 1952; Smith *et al.* 1955). From these studies, however, it was concluded that the rumen wall is not a major site for net P_i absorption. This does not agree with results from recent in vivo studies in sheep measuring P_i absorption by applying the washed and temporarily isolated rumen technique which have shown a close positive linear relationship between rumen P_i concentration and net P_i disappearance (Breves *et al.* 1988; Beardsworth *et al.* 1989). The slope of this regression significantly increased when the experiments were done in P-depleted sheep with plasma P_i concentrations of less than 1 mM. This indicates that rumen P_i transport may be modulated by the transepithelial chemical gradient. Since the normal transmural electrical potential difference also favours passive P_i transport it was postulated that P_i passes the rumen wall by passive diffusion. This could be confirmed by in vitro measurements in Ussing chambers. When the unidirectional flux rates were measured under short-circuit-current conditions no significant net flux could be determined which proved the absence of an active transport system (Breves *et al.* 1988). The involvement of an active transport system had been suggested in an earlier paper based on tissues obtained from a small number of young sheep (Breves *et al.* 1986). Since the unidirectional P_i fluxes could either be enhanced or reduced respectively by setting the serosal side to a positive (+25 mV) or negative (−25 mV) potential, it was concluded that P_i is at least partly transported in the ionized form (Breves *et al.* 1988). Further components of rumen P_i transport have not yet been identified. It should be noted, however, that neither the in vivo nor the in vitro measurements may be used to give a reliable estimate of the quantitative importance of the rumen for overall gastrointestinal P_i absorption.

The omasal wall is characterized by similar transport properties to the rumen (Höller *et al.* 1988*a*) which confirms in vivo experiments in ruminating calves (Edrise & Smith, 1986). In these experiments between 10 and 40% of P entering the omasum were absorbed. In contrast, only a low P absorption of less than 0·5 mM/h was calculated from in vivo studies in sheep (Engelhardt & Hauffe, 1975). A few experiments have been carried out to study P_i absorption across the abomasal wall and it was found that only small amounts of P were either secreted into or absorbed from the abomasum (Smith *et al.* 1955; Poppi & Ternouth, 1979; Sklan & Hurwitz, 1985). In vitro studies have not yet been performed to characterize the transport mechanisms.

Small intestines

It has clearly been shown that as in single-stomach animals, the small intestines are the major site for net P_i absorption in ruminants (Bruce *et al.* 1966/67; Pfeffer *et al.* 1970; Grace *et al.* 1974). In contrast to single-stomach animals, however, little is known about the sites, mechanisms and regulation of intestinal P_i absorption. Care *et al.* (1980) perfused jejunal Thiry–Vella-loops with increasing P_i concentrations and postulated a carrier-mediated facilitated diffusion. Whereas Care *et al.* (1980) increased the P_i concentration to 20 mM and, thus, maintained a physiological concentration range, Scott *et al.* (1984)

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perfused the duodenum and jejunum with P_i concentration up to 50 mM. From the relationship between P_i concentration and P_i absorption they also suggested the involvement of a carrier.

In growing lambs the highest rate of absorption was determined in the mid jejunum, and this could be inhibited by galactose or glucose, indicating a competitive mechanism between P_i and sugars (Scharrer, 1985). Thus, the specificity of the active P_i transport mechanism in lambs is possibly less than in rabbits (Hildmann *et al.* 1982). Recently, P_i uptake by BBMV prepared from enterocytes of the upper small intestine was measured (Shirazi-Beechey *et al.* 1991). An electroneutral P_i uptake was found which depended on the pH gradient between the buffer solutions inside and outside the vesicles. A similar transport system has already been discussed for renal cells and this effect might reflect either changes of the transport properties or pH-dependent changes of the ratio between the mono- and divalent phosphate molecules (Sacktor & Cheng, 1981). The transport capacity was increased during development of the rumen (Shirazi-Beechey *et al.* 1989) and by P depletion (Shirazi-Beechey *et al.* 1991) which might have been due to induction of new transporters. A Na^+–P_i cotransport system could be detected in the ileum only and this could be stimulated by 1,25-$(OH)_2D_3$ (Schneider *et al.* 1987).

Large intestines

Experimental findings on P net absorption from the hind-gut vary over a wide range. When sheep were kept on a daily P intake between 1·0 and 4·1 g P, net absorption ranged between 2 and 30% of the P amount entering the hind-gut (Bruce *et al.* 1966/67; Pfeffer *et al.* 1970; Grace, 1972; Grace *et al.* 1974; Ben-Ghedalia *et al.* 1975, 1982; Greene *et al.* 1983; Breves *et al.* .1985; Théwis & François, 1985; Wylie *et al.* 1985). This could be changed to P net secretion with higher dietary P intakes (Ben-Ghedalia *et al.* 1975; Breves *et al.* 1985).

It is still unclear whether these small amounts of P are transported by active and/or passive transport processes. When the colon and rectum of sheep were continuously perfused with different buffer solutions containing P_i concentrations between 2·0 and 6·5 mM, the measured P_i absorption was equivalent to the theoretical absorption by applying the Nernst equation suggesting a simple diffusion as transport mechanism. At phosphate concentrations between 0·2 and 2·0 mM the measured P_i absorption exceeded the calculated which might be an indicator for an active transport mechanism (Höller *et al.* 1988*b*). However, so far no detailed studies have been performed to characterize the mechanisms possibly involved in phosphate transport in the large intestines.

HORMONAL REGULATION OF P HOMEOSTASIS

From experiments with non-ruminant species it is well known that the regulation of P homeostasis is mainly dependent on both renal P_i excretion and intestinal absorption (Wilkinson, 1976; Neer, 1979; Audran & Kumar, 1985). If an animal is kept on a diet containing less P than it requires, the proportion of dietary P actually absorbed is increased and concomitantly renal P_i reabsorption from the tubuli is also increased in order to minimize urinary P_i output (Audran & Kumar, 1985; Portale *et al.* 1989; Mulroney & Haramati, 1990). In contrast to these species, ruminants do not depend on the kidney as a major route of P regulation, and so far it is not known with certainty whether P_i metabolism in ruminants is regulated primarily at the salivary glands or in the intestines.

Hormonal regulation of Ca and P homeostasis is characterized by common mechanisms and this is mainly achieved by three hormones, i.e. by parathyroid hormone (PTH),

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calcitonin and 1,25-$(OH)_2D_3$ (for reviews, see Neer, 1979; Drüeke & Lacour, 1983; Horst, 1986; Reinhardt *et al.* 1988).

CONTROL OF EPITHELIAL P_i TRANSPORT

It has been known for almost 60 years that both intestinal Ca and P_i absorption can be stimulated by vitamin D (Harris & Innes, 1931; Nicolaysen, 1937). The effect on P_i absorption generally was assumed to be secondary to the improved Ca absorption since in in vitro studies on everted intestinal loops from rats and chicks removal of Ca from the system eliminated the vitamin D effect on P_i transport (Harrison & Harrison, 1961; Morgan, 1969). In contrast to these findings Kowarski & Schachter (1969) have shown, with similar techniques, that vitamin D has a direct and Ca-independent action on the P_i transport system. When Na^+ was removed from the incubation medium P_i transport was inhibited, with no effect on Ca transport, and from this finding individual transport systems were postulated for both electrolytes (Harrison & Harrison, 1963). Additional evidence for independent transport processes was provided by clinical studies on vitamin D-deficient patients suffering from P_i malabsorption with no difference when Ca was either present or absent (Caniggia *et al.* 1968; Nordin, 1973). When these patients were treated with vitamin D, effects on P_i transport could be detected earlier than for Ca (Stamp, 1972). More detailed information about the regulation of intestinal P_i and Ca transport by vitamin D was obtained by applying in vitro ion-flux measurements in Ussing chambers (Ussing & Zerahn, 1951) and by uptake studies in BBMV (Schultz *et al.* 1967).

With both methods it was confirmed that 1,25-$(OH)_2D_3$ stimulates active Ca and P_i transport independently (Danisi & Straub, 1980; Lee *et al.* 1980; Murer & Hildmann, 1981; Hildmann *et al.* 1982; Karsenty *et al.* 1985*a*; Jungbluth & Binswanger, 1989) and that it involves both the classical steroid-receptor-mediated 'slow' genomic effects and 'fast' non-genomic effects (Karsenty *et al.* 1985*b*; Brasitus *et al.* 1986; De Boland & Norman, 1990). Whereas the genomic effects primarily consist of the induction of intracellular Ca-binding-proteins the short-term effects are believed to involve changes in membrane fluidity in response to 1,25-$(OH)_2D_3$ (Rasmussen *et al.* 1979). Additionally it could be demonstrated that 1,25-$(OH)_2D_3$ can also stimulate the paracellular diffusional ion movement at least from the serosal to the mucosal side (Jungbluth & Binswanger, 1989).

Studies on the role of the other important calciotropic hormones PTH and calcitonin have revealed contradictory results with respect to direct effects on Ca and P_i transport (for review, see Drüeke & Lacour, 1983; Lee *et al.* 1986*b*, 1990; Cross *et al.* 1990) and it was suggested that the effects of both hormones may reflect indirect actions. Since PTH and calcitonin are more important in renal and bone Ca–P_i handling, their potential role in regulating intestinal transport will not further be discussed in the present paper.

It should be noted, however, that at least two other hormones might be involved in regulation of intestinal P_i absorption. The full expression of the vitamin-D-dependent Na^+-coupled P_i transport system in immature chick enterocytes required insulin (Peterlik *et al.* 1981) and the maximal stimulation of P_i transport could be achieved when embryonic jejunum was cultured in presence of both 1,25-$(OH)_2D_3$ and triiodothyronine (Cross *et al.* 1986; Cross & Peterlik, 1988).

INTERACTIONS BETWEEN P AND Ca HOMEOSTASIS

Regulation of plasma levels of 1,25-$(OH)_2D_3$ depends on both plasma P_i and Ca concentrations (Audran & Kumar, 1985). In ruminants and non-ruminants the regulatory effects of Ca on plasma 1,25-$(OH)_2D_3$ are mediated by PTH which has been shown to

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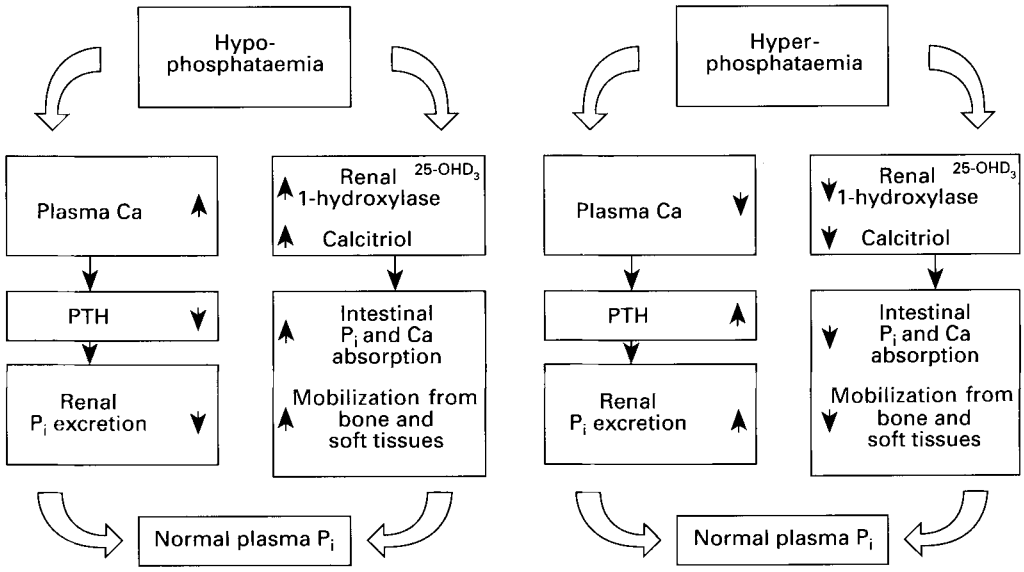

Fig. 1. Adaptational processes to hypo- and hyperphosphataemia. P_i, inorganic phosphate; 25-OHD_3 1-hydroxylase, 25-hydroxycholecalciferol-1-hydroxylase (*EC* 1.14.13.13); calcitriol, 1,25-dihydroxycholecalciferol; PTH, parathyroid hormone.

stimulate the activity of renal 25-hydroxycholecalciferol-1-hydroxylase (*EC* 1.14.13.13; 25-OHD_3 1-hydroxylase; Bilezikian *et al.* 1978; Korkor *et al.* 1985; Reinhardt *et al.* 1988). There is also evidence for direct regulation of this enzyme by Ca (Favus & Langman, 1986). Stimulation of 25-OHD_3 1-hydroxylase by low serum P_i has only been demonstrated in non-ruminants and this obviously does not depend on the presence of PTH (Tanaka & DeLuca, 1973; Fox *et al.* 1978; Gray *et al.* 1983). The effects of low serum P_i on renal 25-OHD_3 1-hydroxylase may be mediated by somatomedins (Gray, 1987).

In ruminants hypophosphataemia is neither associated with any significant changes of plasma 1,25-$(OH)_2D_3$ concentrations nor with any changes of the metabolic clearance or production rate of this hormone, suggesting that adaptational processes to P depletion differ between ruminants and non-ruminants and that in ruminants no changes are involved at the vitamin D level (Breves *et al.* 1985; Maunder *et al.* 1986; Müschen *et al.* 1988; Schröder *et al.* 1990). However, it has been shown recently that, in lactating goats, P depletion resulted in a significant increase in the intestinal 1,25-$(OH)_2D_3$ receptor binding affinity. It still has to be elucidated whether these changes are associated with any effects of intestinal P_i or Ca absorption (Schröder *et al.* 1990). Fig. 1 summarizes the major effects of either hypo- or hyperphosphataemia on adaptational processes in non-ruminant species.

In addition to the stimulatory effects of hypophosphataemia on intestinal P_i absorption, hypercalcaemia develops which can be induced by increased intestinal Ca absorption and/or increased bone mobilization. Hypophosphataemia suppresses PTH secretion and, thus, minimizes urinary P_i losses (Neer, 1979). In addition, intrinsic renal mechanisms may enhance tubular reabsorption and these mechanisms are not related to PTH or vitamin D (Audran & Kumar,1985). The opposite sequence of events occurs in hyperphosphataemia.

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DIETARY P DEFICIENCY IN RUMINANTS: EFFECTS ON MICROBIAL METABOLISM IN THE GASTROINTESTINAL TRACT

Dietary P deficiency is one of the predominant mineral imbalances occurring in areas with extensive ruminant production systems. It may be accompanied by unspecific clinical or subclinical symptoms such as reduction in voluntary feed intake and decreases in growth and reproductive or lactational performance. In response to P depletion, disturbances in bone mineralization may also occur (Cohen, 1975; Field *et al.* 1975; Noller *et al.* 1977; Boxebeld *et al.* 1983; Smith, 1984).

Although reductions in feed intake have been found in many experimental studies the mechanisms by which they are induced are still unknown (Preston & Pfander, 1964; Field *et al.* 1975; Bonilla, 1976; Boxebeld *et al.* 1983). The lowered nutrient supply, as induced by decreased feed intake, may be further amplified by reduced dry matter or organic matter (OM) digestibility (Farries & Krasnodebska, 1972; Field *et al.* 1975; Sevilla & Ternouth, 1980, 1982) and from studies in sheep with duodenal and ileal cannulas it could be demonstrated that in absolute terms the rumen and the hind-gut took about equal shares in the reduction of OM digestion (Breves & Höller, 1987*a*).

This agrees with comparative studies on microbial P requirements when no differences between rumen and hind-gut microbes could be detected (Milton & Ternouth, 1984). In order to ensure an undisturbed microbial metabolism in the rumen minimal P concentrations have to be in a range between 0·7 and 2·6 mM (Hall *et al.* 1961; Chicco *et al.* 1965; Durand *et al.* 1983; Milton & Ternouth, 1984). Komisarczuk *et al.* (1987) have used a continuous rumen incubation system to determine effects on basic fermentative and synthetic processes of rumen microbes brought about by changes in rumen P_i concentrations. By infusion of different buffer solutions P_i concentrations were adjusted to between 1·5 and less than 0·03 mM within the rumen fluid. When the P_i concentration was lowered to 0·1 mM cellulose digestion and volatile fatty acid (VFA) production decreased. Microbial protein synthesis was reduced at P_i concentrations of less than 0·03 mM. These concentrations are significantly lower than those detected in the particle-free rumen fluid of P-depleted sheep, and were accompanied by significant decreases in OM digestibility in the rumen (Breves & Höller, 1987*a*). The discrepancy between in vitro and in vivo results might have been induced by differences in adaptation times to low P_i concentrations. In the in vivo experiments at least 3 weeks were allowed for adaptation to the low-P diet (Breves & Holler, 1987*a*). Only a few in vivo experiments have been performed to study the effects of P depletion on gastrointestinal N metabolism. Decreases in N digestibility were found in sheep (Farries & Krasnodebska, 1972) and the large intestines were the major site for this reduction (Breves & Höller, 1987*b*).

Whereas the nitrogen balance and the ammonia-N:non-NH_3-N (NAN) ratio up to the end of the small intestines were not affected by the P status, significant effects of P depletion on kinetic variables of microbial growth could be demonstrated when $^{15}NH_4Cl$ was used as a label for microbial protein synthesis. Net yield of microbial N was reduced by about 27%; this decreased the microbial N pool in the rumen and correspondingly the flow of microbial N into the proximal duodenum (Lessmann, 1985; Breves & Höller, 1987*b*). In P depletion the proportion of microbial N from the NH_3 pool was lower and the flow of non-microbial NAN into the proximal duodenum was significantly higher. Both findings suggest that P depletion also reduced food protein degradation in the rumen. The efficiency of microbial growth was not affected. These studies were done in sheep which were kept close to maintenance levels. The depressive effects of P depletion on microbial metabolism could be confirmed qualitatively and were even more pronounced quantitatively in

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lactating goats. In these animals P depletion within the third month of lactation decreased the net yield of microbial N by about 60–65%; hence the proportion of milk protein derived from microbial protein was lower (Petri *et al.* 1988).

The mechanisms by which growth depression is exerted have not yet been identified. Since the N:P ratio in microbial dry matter did not change (Breves, unpublished results) insufficient microbial synthesis of essential P compounds might limit microbial growth. Another explanation could be the fact that the method for determination of microbial growth measures net growth, i.e. the difference between gross growth and microbial lysis. Thus, the decreased flow of microbial N into the proximal duodenum might have been induced by an increased microbial lysis. There is no evidence that rumen protozoa are involved in changes of microbial growth. Within 3 weeks after the start of P depletion neither the individual species nor their concentrations changed significantly (Breves, unpublished results).

In conclusion, changes in microbial metabolism in P-depleted sheep and goats have to be considered as a major factor which reduces the endogenous supply of essential amino acids and, thus, is involved in reduced growth and reproductive or lactational performance. This may be amplified under free grazing conditions since P deficiency in plant material often coincides with N deficiency or with low-quality N compounds. Further studies should be carried out to elucidate whether dietary P depletion is also accompanied by changes in intermediary metabolism.

CONCLUSIONS

Secretory and absorptive processes for P_i along the gastrointestinal tract are characterized by major differences between ruminant and non-ruminant species. The high salivary P_i secretion in ruminants is well documented and, at least in sheep, the end-pieces of the parotid glands were found to be the major site for P_i secretion. Little is known about the transport processes involved and how the uphill P_i transport is regulated is still unknown.

Numerous experiments have been performed in single-stomach animals to identify epithelial P_i transport in the small intestines and at least two mechanisms were found: (1) a passive non-saturable transport and (2) a secondary active Na^+-coupled mechanism which is controlled by 1,25-$(OH)_2D_3$.

It is still unknown whether similar processes are present in the small intestines of ruminants. Recent findings suggest that in sheep P_i uptake at the lumen side might be controlled by the pH gradient across the membrane. Although most of P_i is absorbed across the small intestinal wall, the rumen and omasum have been shown to be a potential site for net P_i absorption, and from in vitro studies it was concluded that, in both organs, P_i is absorbed by passive diffusion. In single-stomach animals hypophosphataemia enhances renal production of 1,25-$(OH)_2D_3$ and, thus, intestinal absorption of Ca and P_i which results in hypercalcaemia. In sheep and goats hypophosphataemia also induces hypercalcaemia without any effect on concentration or production rate of 1,25-$(OH)_2D_3$. Recent studies, however, have shown that goats at least adapt at the intestinal 1,25-$(OH)_2D_3$ receptor level, since in P depletion the affinity of these receptors significantly increased. The physiological significance of these changes is not yet understood.

Rumen microbial metabolism is affected by long-term dietary P depletion. When rumen P concentrations are lower than 3 mM OM digestibility and microbial protein synthesis decrease significantly, and these changes may be of primary importance when P-deficient animals under free grazing conditions decrease milk production, growth or lactational performance.

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Thus, it may be speculated from the teleological point of view that ruminants have developed high salivary P_i secretion in order to maintain rumen microbial metabolism as long as possible independent from dietary P supply.

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