

Ontogenesis of epithelial phosphate transport systems in goats

KORINNA HUBER,¹ UTA ROESLER,¹ ALEXANDRA MUSCHER,¹ KATHRIN HANSEN,¹ IRKHAM WIDIYONO,² ERNST PFEFFER,³ AND GERHARD BREVES¹

¹*Department of Physiology, School of Veterinary Medicine Hannover, D-30173 Hannover;* ³*Institute of Animal Nutrition, Rheinische Friedrich-Wilhelm-University, D-53115 Bonn, Germany;* and ²*Fakultas Kedokteran Hewan, Universitas Gadjah Mada, Sekip Unit II, Yogyakarta, Indonesia*

Submitted 17 June 2002; accepted in final form 8 October 2002

Huber, Korinna, Uta Roesler, Alexandra Muscher, Kathrin Hansen, Irkham Widiyono, Ernst Pfeffer, and Gerhard Breves. Ontogenesis of epithelial phosphate transport systems in goats. *Am J Physiol Regul Integr Comp Physiol* 284: R413–R421, 2003. First published October 10, 2002; 10.1152/ajpregu.00357.2002.—The rapid development of precocial goats in the first weeks after birth requires an adequate adaptation of phosphate transport systems to maintain the P homeostasis at each developmental stage. Here we examined the age-related development of Na⁺-P_i transport systems in small intestines, kidneys, and parotid glands of goats. Kinetic parameters were determined by brush-border membrane vesicle uptake studies, and relative expression of NaPi type II mRNA and protein was recorded by molecular biological methods. High intestinal P_i transport capacity was already present on the first day of life. Within the first 3 wk of life there seemed to be a change in the type of Na⁺-dependent P_i transporter, and NaPi IIb was expressed increasingly up to the fifth month of life. Renal Na⁺-P_i transport capacity was also high at birth, and this was associated with high expression levels of NaPi IIa mRNA, indicating the important role of this transporter for renal P_i reabsorption. At weaning an increase in both intestinal and renal Na⁺-P_i transport balanced the increasing requirements for P_i to establish the endogenous P_i cycle. Salivary P_i concentration and parotid NaPi II mRNA rose markedly to guarantee an adequate P_i supply for rumen microbes. We concluded that the high demand for P_i in young goats was assured by high basal Na⁺-P_i transport capacity of small intestines and kidney expressed continuously during ontogenesis.

ontogenesis of sodium-phosphate transport; ruminants; small intestine; kidney; parotid gland; NaPi type II cotransporters

DEVELOPING YOUNG ORGANISMS have a high requirement for phosphorus (P) for the mineralization of the skeleton and maintenance of metabolism and growth. Newborn precocial species such as goats are able to stand up within a few hours after birth, which implies a well-developed bone mineralization and an adequate energy metabolism for mobility and ther-

moregulation. In contrast, altricial species such as mice or rabbits do not have a comparable demand for P due to their stage of maturation at birth. To meet the high P requirement of developing goats, there must be adequate intestinal P_i absorption and renal P_i reabsorption as well. In adult goats epithelial P_i transporters in the apical membranes in the small intestines and in the kidney are characterized structurally and functionally. In the jejunum, a Na⁺-dependent P_i transport has been characterized that was molecularly based on a NaPi type IIb cotransporter (9, 14). In the kidney cortex, P_i reabsorption is mediated by a NaPi type IIa cotransporter, but its quantitative proportion on overall renal Na⁺-dependent P_i transport is still unknown (15). When young preruminant goats are gradually adapted to a solid diet, the endogenous P_i cycle will start to develop because of the increasing ability of the salivary glands, particularly the parotid glands, to transport P_i from blood to saliva. There is some experimental evidence that P_i transport in the salivary glands is Na⁺ dependent and basolateral localized (19), but the exact cellular mechanisms and localization of transporters are not yet fully understood. Salivary secretion is stimulated by mechanical as well as chemical stimuli of mouth and rumen mucosa receptors when intake of dietary roughage begins.

When the endogenous P_i cycling is established, P_i from intestinal absorption and renal reabsorption is recycled by concentrating P_i in the saliva and secreting it with the saliva into the rumen. Thus, at a continuous salivary secretion rate of 6–16 l/day in adult small ruminants with a mean salivary P_i concentration of 25 mmol/l, 2.4–6.4 g P/day flows into the rumen. High salivary concentrations of both P_i and bicarbonate prevent ruminal acidification by short-chain fatty acids (SCFA), which are the major end products of microbial fermentation. Furthermore, P_i is necessary to build up microbial cell walls, to form energy-rich phosphates, and to synthesize DNA for microbial growth and metabolism (7). Thus

Address for reprint requests and other correspondence: K. Huber, Dept. of Physiology, School of Veterinary Medicine Hannover, Bischofsholer Damm 15/102, D-30173 Hannover, Germany (E-mail: korinna.huber@tiho-hannover.de).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

the development of the endogenous P_i cycle can be considered a strategy to ensure rumen microbial homeostasis to provide adequate amounts of SCFA as energy sources and high-quality microbial protein to the host organism.

There must be a sufficient postnatal development of P_i transport systems to guarantee an adequate P_i homeostasis at each stage of the developmental process. As this is poorly understood, it was the aim of this study to characterize the ontogenesis of the epithelial P_i transport systems in goats. The age groups studied spanned four stages of development: suckling kids in the 1st week of life, suckling kids in the 4th to 5th week of life, weaning kids in the 8th to 11th week of life, and ruminating goats in the 4th to 5th month of life. This corresponds to the four food intake periods: colostrum/first milk, pure milk, milk and first crude fiber, and milk-free, adult food. Expression of P_i transporters was examined in the jejunum, the kidney, and the salivary gland at the molecular and functional levels. For the first time, a NaPi type II transporter was demonstrated in the parotid glands.

MATERIALS AND METHODS

Animals and Feeding

Male kids of the Saanen-type breed Weisse Deutsche Edelziege were used for the experiments. The kids were between 1 and 150 days of age (see Table 1 for detailed information on groups, age, feeding and housing, and body weights). Plasma and saliva samples were taken to determine P status of the kids in each age group. Saliva was collected by placing a sponge close to the orifice of the parotid duct for a few minutes according to Boehnke et al. (3). General care and handling of the animals were approved and supervised by the Animal Welfare Commissioners of the University of Bonn and the School of Veterinary Medicine Hannover in accordance with the German Animal Welfare Law.

Animals were killed by stunning using a captive bolt pistol and bled from the carotid arteries. Kidneys, parotid glands, and midjejunal segments were obtained within 3–5 min after slaughter. After rinsing with ice-cold saline (0.9% NaCl wt/vol), parotid glands, jejunal mucosa, and kidney cortexes were frozen in liquid nitrogen and stored at -80°C for structural and functional analyses.

NaPi II-Specific RT-PCR in the Parotid Gland

RT-PCR was performed using poly(A)⁺ RNA isolated from parotid glands. Using 500 U Moloney mouse leukemia virus

(MMuLV) RT (MBI Fermentas, Vilnius, Lithuania), 5 μg poly(A)⁺ RNA was transcribed into cDNA. PCR was performed at the following reaction conditions: 1 \times reaction buffer (200 mmol/l Tris·HCl, pH 8.4, 500 mmol/l KCl), 0.4 mmol/l each dNTP, 1.5 mmol/l MgCl₂, 2.5 U *Taq* polymerase (GIBCO BRL), 20 pmol of each primer, and 1.5 μl template in a total volume of 25 μl . Using a Mastercycler gradient (Eppendorf, Germany), 34 cycles were run with denaturing for 30 s (after an initial denaturing for 2 min at 94 $^{\circ}\text{C}$), annealing for 1 min at 60 $^{\circ}\text{C}$, and elongation for 2 min at 72 $^{\circ}\text{C}$. Finally, there was an elongation time of 15 min at 72 $^{\circ}\text{C}$. Specific primers were derived from bovine kidney cell line NaPi IIb mRNA sequence (GenBank accession no. X81699): sense 5'-atgggtgctctctactgctg-3' (nucleotides 609–629) and antisense 5'-tggggtcatagcagacgtgaa-3' (nucleotides 1406–1429). A band of 819 bp could be detected by agarose gel electrophoresis, which was identified by cloning and sequencing (sequenced by Agowa, Berlin, Germany).

Northern Blot Analyses

Semiquantitative detection of specific NaPi II mRNAs of jejunum, kidney, and parotid gland was performed as described in detail elsewhere (9). In short, total RNA from kidney cortex and poly(A)⁺ RNA from parotid gland and jejunal mucosa were fractionated in 1.0% formamide/agarose gels and transferred by capillary blotting onto nitrocellulose membranes. Fixed mRNAs were hybridized to radioactively labeled NaPi II- and β -actin-specific probes. The membranes were analyzed after exposure to a phosphorus imager screen for 2–4 h with a phosphorus imager system (Bio-Rad). The relative amounts of specific mRNA were quantified by reference to β -actin as an internal standard using the quantification software Quantity One (Bio-Rad).

Western Blot Analyses

Semiquantitative detection of NaPi IIb protein in brush-border membranes (BBMs) of jejunal enterocytes was performed as described elsewhere (9). The anti-NaPi IIb antibody was kindly provided by Prof. Dr. J. Biber and Prof. Dr. H. Murer, Institute of Physiology, University of Zürich-Irchel, Zürich, Switzerland. Bands were analyzed quantitatively using the Quantity One software.

Preparation of BBM Vesicles and Uptake Measurements

BBM vesicles (BBMV) were prepared from renal and jejunal epithelia with a modified Mg²⁺-EGTA precipitation method, and P_i uptake into BBMV was quantified using the rapid filtration technique as described for jejunum and kidney (14, 15). Enrichment of the BBM preparations was determined by marker enzyme activities [alkaline phos-

Table 1. Age, feeding, body mass, P intake, and plasma P_i concentrations of kids

Group	Age, days	n	Feeding/Housing	Body Mass, kg	Saliva P_i , mmol/l	Plasma P_i , mmol/l
1–7 day	1–7	8	colostrum, milk/kids kept with their mothers	3.5 \pm 1.3	not measurable	2.2 \pm 0.5*
4–5 wk	26–31	4	milk from the mother/kids kept with their mothers	6.4 \pm 0.9	8.7 \pm 6.1*	3.2 \pm 0.2†
8–11 wk	51–74	4	bulked milk of all mothers/kids kept individually in boxes with straw bedding	14.9 \pm 0.6	39.9 \pm 6.1†	3.1 \pm 0.2†
4–5 mo	~150	6	concentrate and straw/goats kept together	31.6 \pm 4.9	35.5 \pm 7.2†	1.5 \pm 0.2‡

Values are given as means \pm SD. Significance levels were applied to values in columns: $P < 0.01$ (* vs. †); $P < 0.05$ (* vs. ‡); $P < 0.001$ († vs. ‡). Values were tested by 1-way ANOVA/Tukey's *t*-test (Software Graphpad prism 3.0, San Diego, CA).

phatase (AP) for BBM; Na⁺-K⁺-ATPase for basolateral membranes]. The used standardized preparation method resulted in nearly pure BBM without endoplasmic contaminations (14). Protein concentration was assayed utilizing the DC method from Bio-Rad (Hercules, CA). Vesicular P_i transport was analyzed in the presence of an inwardly directed Na⁺ gradient [extravesicular buffer (in mmol/l): 100 NaCl, 100 mannitol, 10 HEPES-Tris, pH 7.4; intravesicular buffer (in mmol/l): 100 KCl, 100 mannitol, 10 HEPES-Tris, pH 7.4]. To calculate the P_i diffusion across the BBM, P_i uptake was measured without an inwardly directed Na⁺ gradient at an extravesicular P_i concentration of 0.1 mmol/l. The diffusional part of P_i uptake was subtracted from the total P_i uptake, resulting in net Na⁺-dependent P_i transport. Kinetic parameters V_{max} (nmol·mg protein⁻¹·10 s⁻¹) and K_m (mmol/l) for Na⁺-dependent P_i transport in kidney and jejunum were calculated from the Michaelis-Menten kinetic for P_i uptake into the BBMV (14).

Determination of Plasma and Salivary P_i

P_i concentration in plasma and saliva was determined colorimetrically using the vanadate-molybdate method (10).

Statistics

Values were given as means ± SD or SE, with n = number of animals (Tables 1 and 2). Significance of differences between plasma and saliva P_i concentrations and kinetic parameters of Na-P_i transport was tested by one-way ANOVA and as a posttest by Tukey's *t*-test (software Graphpad prism 3.0, San Diego, CA; www.graphpad.com). *P* values of <0.05 were set to be significant. Results shown in Figs. 1–3 are given as individual data to illustrate the developmental processes in more detail. Means of blot data (Figs. 1–3, absolute values) are given in Table 2; results of statistical analysis are

given in Table 3. All immunodetection and hybridization experiments were performed at least in duplicate. Data of all performed mRNA and protein blots were related to the respective 1- to 7-day values as a 100% basis (Table 2, relative values). All NaPi transport data were correlated with age by linear regression analysis (software Graphpad prism 3.0) (Table 3). For approximation of data in nonruminating and ruminating goats, the absolute and relative values were used, respectively.

RESULTS

Developmental Stages of Animals

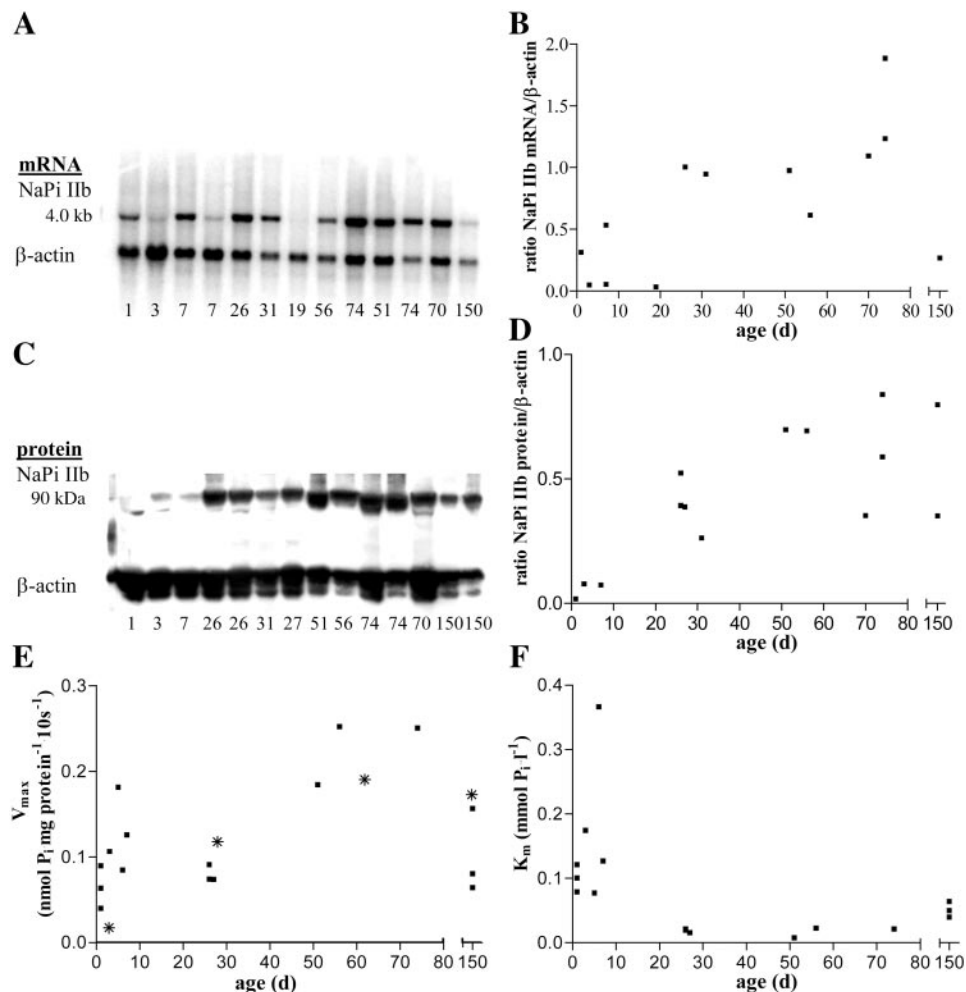
The goat kids were allowed to suckle their mothers for the first week of life (group: 1–7 days). Therefore, they were nourished exclusively with colostrum and milk from the first week of lactation. Four- to five-week-old goats (group: 4–5 wk) were also nourished with milk from later lactation by suckling their mothers. The kids were kept with their mothers on straw bedding and so were able to begin eating small amounts of their mothers' straw, hay, or concentrate feed. Eight- to eleven-week-old goats (group: 8–11 wk) were fed ad libitum with milk collected from the mother herd. Because the kids were kept on straw bedding, they ingested straw in amounts that could not be measured quantitatively. Sporadic rumination and measurable salivary secretion were observed in these animals, indicating the onset of weaning. Furthermore, at slaughter the rumen was filled with straw particles. Four- to five-month-old goats (group: 4–5 mo) kept on a concentrate/straw

Table 2. Relative amounts of NaPi II mRNA and protein and kinetic parameters of Na⁺-dependent P_i transport in goat jejunum and kidney

Group	mRNA, % (NaPi II/β-actin)	n	Protein, % (NaPi II/β-actin)	n	V _{max} , nmol·mg ⁻¹ ·10 s ⁻¹	n	K _m , mmol/l	n
<i>Jejunum</i>								
1–7 day	100 (0.24 ± 0.23)	5	100 (0.06 ± 0.03)	3	0.10 ± 0.05*	6	0.11 ± 0.04*	6
4–5 wk	278.2 ± 229.2 (0.66 ± 0.54)	3	675.3 ± 166.6 (0.39 ± 0.11)	4	0.08 ± 0.01*	3	0.02 ± 0.003†	3
8–11 wk	442.4 ± 208.8 (1.16 ± 0.47)	5	1,146.0 ± 348.5 (0.63 ± 0.18)	5	0.23 ± 0.04†	3	0.02 ± 0.008†	3
4–5 mo	65.5 ± 26.5 (0.27)	3	1,030.1 ± 610.9 (0.57)	3	0.11 ± 0.04*	3	0.05 ± 0.01†	3
<i>Kidney</i>								
1–7 day	100 (0.56 ± 0.14)	8			3.57 ± 1.46*	7	0.31 ± 0.17	7
4–5 wk	156.3 ± 35.0 (0.67 ± 0.27)	3			3.59 ± 1.66*	4	0.20 ± 0.11	4
8–11 wk	89.9 ± 18.3 (0.36 ± 0.06)	5			6.04 ± 1.48†	4	0.53 ± 0.17	4
4–5 mo	56.8 ± 20.0 (0.36 ± 0.18)	6			2.73 ± 0.46*	6	0.49 ± 0.14	6

Values are given as means ± SD; n = no. of animals. Relative mRNA and protein amounts are given in % of 1–7 day value (100%) and also in parentheses as NaPi II/β-actin ratio as derived from blot data shown in Figs. 1–3. For relative mRNA and protein amounts in %, each recorded measurement reading was the mean of 1–3 repeats and was related to the 1–7 day group as 100% basis. Therefore, the given means ± SD represent the interindividual variation. Kinetic data were tested by 1-way ANOVA compared with Tukey's test, assuming a Gaussian distribution of the values. Significant statistical differences between kinetic parameters of age groups compared with 1–7 day goats were indicated by different symbols within each column on a significance level of at least *P* < 0.05. Further statistical calculations are given in Table 3.

Fig. 1. Jejunal Na^+ -dependent P_i transport during ontogenesis in goats. NaPi IIb mRNA [Northern blot (A) and quantification (B)] as well as NaPi IIb protein expression [Western blot (C) and quantification (D)] in goat jejunum was shown as influenced by aging. Detailed information about the Northern and Western analyses is given in MATERIALS AND METHODS. The NaPi II/β-actin ratio accounts for the relative amounts of specific mRNA and protein, respectively. Na^+ -dependent P_i uptake into isolated brush-border membrane vesicles (BBMV) was determined at phosphate concentrations ranging from 0.05 to 3 mmol/l under initial rate conditions (10 s). Kinetic parameters V_{\max} (E) and K_m (F) were derived at every developmental stage using the Michaelis-Menten equation. *E*: *means of relative amounts of NaPi IIb protein of each age group (see also D) to correlate the transport capacity and specific NaPi IIb protein levels (see DISCUSSION). In all graphs, each point represents a single animal (for means \pm SD of all age groups and statistics, see RESULTS).



diet expressed physiological rumen activity and an adequate salivary secretion.

Plasma and Saliva P_i Concentrations During Ontogenesis

During the first 4 wk of life, plasma P_i concentrations increased significantly from 2.2 to 3.2 mmol/l and remained constant at this level until the 8th to 11th week. At the fifth month, plasma P_i concentrations markedly decreased to 1.5 mmol/l in ruminating growing goats (Table 1). Neither saliva sampling nor measurement of salivary P_i concentration was possible until the third week of life. At this age, salivary P_i concentration was as high as the plasma P_i concentration and increased continuously up to the 8th to 11th week of life, peaking at ~ 40 mmol/l. No further increase occurred (see Fig. 3C) in the 5-mo-old goats.

Jejunal Na^+ -Dependent P_i Transport During Ontogenesis

NaPi IIb mRNA expression in the jejunum of nonruminating goats (1–7 days to 8–11 wk) increased during aging up to the 8th to 11th week of life (Fig. 1, A and B; Tables 2 and 3). Simultaneously, NaPi IIb protein expression as well as V_{\max} increased during this devel-

opmental stage (Fig. 1, C–E; Tables 2 and 3). In ruminating goats (8–11 wk to 4–5 mo of age), mRNA levels decreased with aging. Although the protein expression level remained as high as in that of 8- to 11-wk-old animals, V_{\max} decreased during aging. Interestingly, transport capacity (V_{\max}) of Na^+ -dependent P_i transport during the first days of life was as high as in 4–5 mo of age. There was a distinct variation of K_m values depending on age. K_m values decreased from the 1st until the 11th week of life (Fig. 1F; Tables 2 and 3), while in ruminating goats there was a significant increase of K_m .

Renal Na^+ -Dependent P_i Transport During Ontogenesis

NaPi IIa mRNA expression level did not change in the nonruminating goats (Fig. 2, A and B; Tables 2 and 3). NaPi IIa protein expression could not be investigated; no specific antibody against goat renal NaPi IIa is as yet available. The transport capacity of Na^+ -dependent P_i transport slightly increased (Fig. 2C; Tables 2 and 3), but the affinity of the transport slightly decreased (K_m values increased; Fig. 2D; Tables 2 and 3). In ruminating goats NaPi IIa mRNA levels decreased, and, simultaneously, V_{\max} also de-

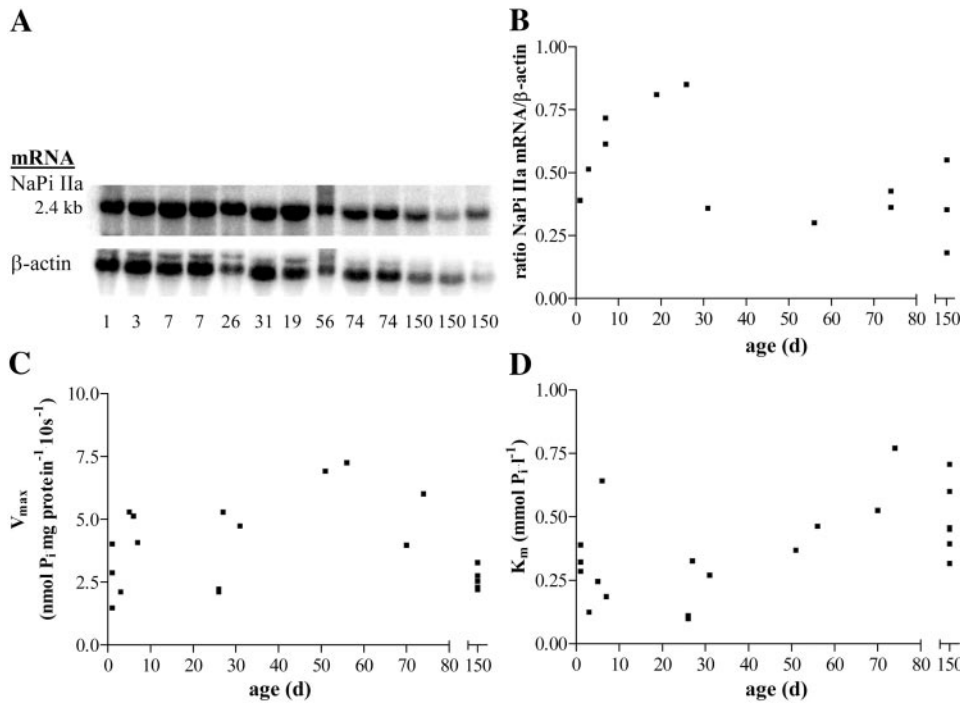


Fig. 2. Renal Na⁺-dependent P_i transport during ontogenesis in goats. NaPi IIa mRNA expression [Northern blot (A) and quantification (B)] was shown as influenced by aging. Detailed information about the Northern analysis is given in MATERIALS AND METHODS. The ratio of NaPi II/β-actin accounts for the relative amounts of specific mRNA. Na⁺-dependent P_i uptake into isolated BBMVs was determined at phosphate concentrations ranging from 0.05 to 3 mmol/l under initial rate conditions (10 s). Kinetic parameters V_{max} (C) and K_m (D) were derived at every developmental stage using the Michaelis-Menten equation. In all graphs, each point represents a single animal (for means ± SD of all age groups and statistics, see RESULTS).

creased. K_m values were not affected during this developmental stage.

P_i Transport in the Parotid Gland During Ontogenesis

Using RT-PCR, a NaPi II-specific fragment was cloned from goat parotid gland (Fig. 3D). The deduced amino acid sequence showed ~98% homology to the bovine NaPi IIB sequence (accession no. X81699) and ~66% homology to renal NaPi IIA of sheep (accession no. AJ001385) (Table 4). The subtype of this NaPi II has not yet been determined. Using this PCR fragment as a probe in Northern analysis of goat parotid gland mRNA, a specific band could be detected at ~1.9 kb (data not shown). Therefore, this NaPi II-specific fragment was used to study its expression in the parotid glands of growing goats. Expression of NaPi II mRNA started at the fourth week of life (the NaPi IIB mRNA/β-actin ratio at 4–5 wk: 0.12) and increased continuously up to the fifth month (the NaPi IIB mRNA/β-actin ratio at 8–11 wk: 0.69 ± 0.09; at 4–5 mo: 2.31 ± 0.73) (Fig. 3, A and B; Table 3). Between the 3rd and 11th week of life, the increase of salivary P_i concentrations was accompanied by an increase of NaPi II mRNA expression. Older ruminating goats still expressed higher mRNA levels without a further increase in salivary P_i concentration (Fig. 3C). In contrast, with PCR, specific bands could also be detected in the 1-wk-old parotid glands (Fig. 3D), indicating that Northern sensitivity was insufficient for detection of NaPi II mRNA at this developmental stage.

DISCUSSION

The high P_i demand of newborn precocial species such as goats must be balanced by adequate intestinal

P_i absorption and renal P_i reabsorption to maintain a positive P balance during ontogenesis. Na⁺-dependent P_i transport in jejunum, kidney, and parotid gland was studied on functional as well as on molecular levels from the first day until 5 mo of age. The ontogenic changes documented here are among the so-called “specific changes,” i.e., changes in the turnover rate, the site density of transporters, the affinity constant, and/or the expression of different isoforms of transporters (13). Nonspecific ontogenic changes, such as changes in epithelial surface area, proliferation and migration of epithelial cells, phospholipid composition and fluidity of plasma membrane, and paracellular permeability, are also very relevant mechanisms of ontogenetic development (13). Additionally, nephron loss in the kidneys from the juxtamedullary region, changes in proximal tubular cells, such as decreased numbers of mitochondria and reduced basolateral Na⁺-K⁺-ATPase activity, could also contribute to the decrease of renal P_i reabsorption capacity during aging (12). These mechanisms were not determined in the present study but have to be kept in mind as additional factors involved in adaptational processes. Ontogenic changes are normally irreversible as opposed to adaptational changes in response to external factors such as P supply. Mature polarized epithelial cells are an essential prerequisite for transport processes in the context of ontogenic changes. Therefore, expression of transporters is strongly correlated to brush border formation (5, 18).

Establishment of the Endogenous P_i Cycle During Ontogenesis

Nonruminating goats. The increase of salivary P_i level and NaPi II mRNA expression starting at the

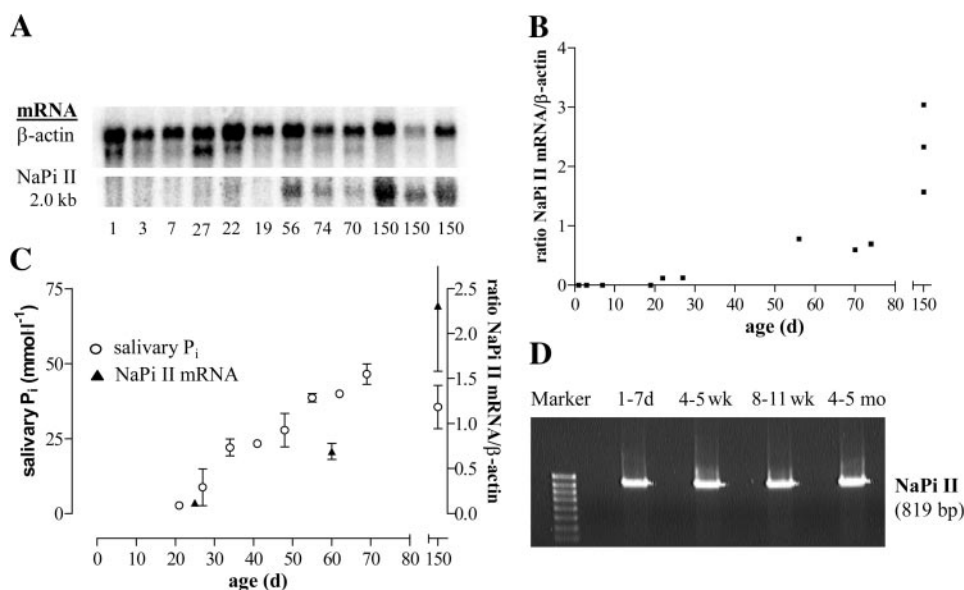


Fig. 3. P_i transport in the parotid gland during ontogenesis in goats. NaPi II mRNA expression [Northern blot (A) and quantification (B)] is shown as influenced by aging. Detailed information about the Northern analysis is given in MATERIALS AND METHODS. The NaPi II/ β -actin ratio accounts for the relative amounts of specific mRNA. Instead of functional data about P_i transport in the parotid gland, P_i concentrations in the saliva are given for each age group correlated to NaPi II expression (mean \pm SD of each age group; values are given in RESULTS; C). As the 1st sampling of saliva was possible at 3 wk of age, there were no earlier determinations of P_i concentrations. D: specific amplification of NaPi II-specific cDNA fragments in parotid glands with RT-PCR, where each band is the result of 1 animal per age group. Because specific NaPi II fragments were also detected in the 1st week of life, the sensitivity of Northern blot is insufficient for examination of NaPi II mRNA expression at this developmental stage. The NaPi II-specific primers used were derived from the NaPi IIb mRNA sequence of bovine renal epithelial cell line (accession no. X81699).

third week of life indicates that endogenous P_i recycling has begun even though the rumen is still poorly developed due to the milk diet and before stimulation of salivary secretion rate by solid feed intake. This indicates the presence of at least in part genetically determined NaPi II expression in the parotid gland. At the 8th to 11th week of life, salivary P_i concentrations had reached the levels of adult goats. Although the intake of solid feed was not measured in this study, it must be assumed that due to its increasing intake at this age, salivary secretion was strongly stimulated mechanically. The ability to concentrate P_i in the saliva, possibly by a Na^+ - P_i type II cotransporter, in connection with the high salivary secretion rate demonstrated the onset of the endogenous P_i cycle at this developmental stage. Surprisingly, the enhanced outflow of P_i from the plasma P_i pool into the growing ruminal compartment did not affect plasma P_i concentrations, which were identical to those of 4- to 5-wk-old milk-fed goats. Therefore, intestinal P_i absorption and renal P_i reabsorption must have been adjusted to meet the P_i needs for endogenous recycling. The increase in V_{max} of Na^+ - P_i transport during the nonruminating period up to weaning in the jejunum as well as in the kidney strengthened this assumption. Thus the nonruminant period seemed to be an important phase for maturation of intestinal P_i transport. This is in close agreement with the progressive intestinal alteration of mucosal morphology and cell migration velocity observed in suckling lambs from the first to the eighth

week of life. While migration rate of cells was highest in newborn lambs, resulting in a short epithelium renewal time of 2–4 days, the migration rate decreased during nonruminating period and renewal time was prolonged (2). This longer life span of enterocytes could contribute to the increasing Na^+ - P_i transport expression during suckling period. Additionally, the bioavailability of P_i in the solid feed could be reduced compared with that in milk (1). Thus the increase in the intestinal P_i transport capacity could also be due to a stimulation of P_i transporter expression in response to restriction of available dietary P_i in terms of an adaptational process as in adult goats (9).

In newborn goats, K_m values of intestinal P_i transport were significantly higher than in all other age groups, indicating a lower affinity for P_i at this developmental stage, although transport capacity was as high as in ruminating goats. Because in adult goats NaPi IIb protein expression is correlated with transport capacity (9), newborn goats should express a lower transport capacity due to low NaPi IIb protein content in the BBM. Existing high transport capacity implies that in the first week another type of Na^+ -dependent P_i transporter could be present in the jejunum to protect the newborn kid from insufficient intestinal P_i absorption until ontogenetic maturation of the NaPi IIb-mediated Na^+ - P_i transport. From the 4th until the 11th week of life, jejunal Na^+ - P_i transport is mediated by NaPi IIb in young goats.

Table 3. Correlation of NaPi transport data with age by linear regression analysis ($y = a + bx$) according to ruminating status of animals

	Data	R ²	a	Slope b	P Value
<i>Jejunum</i>					
Nonruminating	mRNA blot	0.67	0.16 ± 0.16	0.016 ± 0.004	<0.001
	protein blot	0.67	0.11 ± 0.08	0.008 ± 0.002	<0.01
	V _{max}	0.57	0.08 ± 0.02	0.002 ± 0.001	<0.01
	K _m	0.55	0.10 ± 0.02	-0.0016 ± 0.0005	<0.05
Ruminating	mRNA %	0.52	679 ± 158	-3.912 ± 1.424	<0.05
	protein %				NS
	V _{max}	0.69	0.30 ± 0.05	-0.0012 ± 0.0004	<0.01
	K _m	0.82	-0.01 ± 0.01	0.0004 ± 0.0001	<0.01
<i>Kidney</i>					
Nonruminating	mRNA blot				NS
	V _{max}	0.29	3.26 ± 0.58	0.038 ± 0.016	<0.05
	K _m	0.28	0.24 ± 0.06	0.004 ± 0.002	<0.05
Ruminating	mRNA %	0.50	116 ± 15.8	-0.396 ± 0.132	<0.05
	V _{max}	0.70	8.44 ± 1.06	-0.037 ± 0.009	<0.01
	K _m				NS
<i>Salivary gland</i>					
Nonruminating	mRNA blot	0.88	-0.08 ± 0.06	0.011 ± 0.001	<0.001
Ruminating	mRNA blot	0.76	-0.56 ± 0.62	0.019 ± 0.005	<0.05

Values are means ± SE for n goats; n values are given in Table 2. Nonruminating goats, 1–7 day to 8–11 wk; ruminating goats, 8–11 wk to 4–5 mo; mRNA and protein blots, data (NaPi II/β-actin ratio) from blots shown in Figs. 1–3 and Table 2; mRNA and protein %, data (%) given in Table 2 related to 1–7 day value as 100%; NS, not significant. Values for salivary NaPi II mRNA expression are given in the text.

The magnitude of renal P_i transport capacity was much higher in goats than in monogastric species such as rats at corresponding developmental stages (17), indicating that a higher renal P_i reabsorption capacity was expressed even in newborn goats and persisted during aging. Fetal sheep kidney development was finished at 18–90% of gestation (21), resulting in mature glomeruli at day 140 of gestation (8). If this is also true for goat kidney development, the prerequisite was given for the expression of Na⁺-coupled P_i transport across the apical membranes of tubular cells in newborns. Because high V_{max} values result in a renal P_i reabsorption capacity of ~99% in adult goats (20), the comparable high P_i transport capacities in newborn and young goats imply the

early availability of this high P_i reabsorption capacity. Increasing P_i transport capacity in nonruminating goats could also partly depend on the increasing glomerular filtration rate (GFR) observed in developing sheep (11). Higher GFR resulted in an increase of glomerular filtrated P_i, and to maintain the high tubular reabsorption capacity, P_i transport expression has to be enhanced.

Ruminating goats. Four- to five-month-old goats fully adapted to solid feed were characterized by significantly reduced plasma P_i concentrations due to their decreasing metabolic demands (16). P_i transport capacities in jejunum and kidney were significantly reduced compared with nonruminating goats. This has also been described in monogastric animals

Table 4. Deduced amino acid sequence of the NaPi II-specific cDNA fragment of parotid gland of goats created by RT-PCR compared with corresponding NaPi II sequences of other ruminants

SMVSSGLLEVSSAIP I IMGSNIGTSVTNTIVALMQAGDRDFRRAFAGATVHDFCNWLSVLVLLP	
SMVASSLLPVHAAIPI IMGANIGTSITNTIVALMQAGDRKEFRRAFAGATVHDFFNWLSVLVLLP	
MVASSLLPVNAAIPI IMGANIGTSITNTIVALMQAGDRKEFRRAFAGATVHDFFNWLSVLVLLP	
LEAATGYLHHITRLVVASFNIRGRDAPDLLKI I IEPFTKLI IQLDKSVITSLASGDESLRNHSL	
LEAATGYLERLTNLVVESPHFKNGEEAPELLKVI TDPFTKLI IQLDKSILNQIAMNDESVQNKSM	
LEAATGYLERLTNLVVESPHFKNGEEAPELLKVI TDPFTKLI IQLDKSILNQIAMNDESVQNKSM	
IRVWCYPNPTEVPTPMPRAEANTSRMLRNATL - - - - - EKCNIHFVDTGLPDLA	
IKIWCKTFTNVTERNVTVPSPENCTSPSLCWTGGLYTWTIKNVTYKENIAKQCQHFVNFNLSDAI	
IKIWCKTFTNVTERNVTVPSPENCTSPSLCWTGGLYTWTIKNVTYKEDIAKCRHIFVNFNLSDAI	
VGLILLAGSLALLCTCLILLVKMLNSLLKGVAKVIQKVINTDFPTPTWATGYFAMVVGASMTF	
VGTILLITSLILCTCLILIVKLLGSLVLRGQVA AVIKKTINTDFPYPPFS-VTGYLAILVAGMTF	
VGVILLITSLILCTCLILIVKLLGSLVLRGQVA AVIKKTINTDFPYPPFGWVTGYLAILVAGMTF	
VVQSSSVFTSAITPLI NaPiIIa/sheep (accession no. AJ001385)	
IVQSSSVFTSAMTPLI NaPiIIb/cow (accession no. X81699)	
IVQSSSVFTSAMTP NaPiIII/goat	

Caprine NaPi II expressed 98% homology to bovine NaPi IIb and 66% to ovine NaPi IIa.

at comparable developmental stages (4, 17). It is unclear whether the reduced intestinal P_i absorption or the diminished renal P_i reabsorption is a primary reason for establishing lower plasma P_i levels. Likewise, it is unclear which mechanisms regulate these changes. Hormones such as thyroid hormone and growth hormone could have a strongly stimulating effect on Na^+P_i transport processes and are closely involved in the postnatal development of mammals (1). Other hormones and changes in dietary P_i supply and in the extent of cellular P_i -consuming processes may also play important roles in the complex regulation of P homeostasis during ontogenesis.

In these ruminating goats, NaPi IIb expression was studied during ontogenesis because this transporter is mainly responsible for jejunal Na^+P_i transport in adult goats (9). In 4- to 5-mo-old goats, NaPi IIb protein level was as high as in 8- to 11-wk-old goats, but the specific mRNA level was significantly reduced. This could be for several reasons. First, because the life span of enterocytes is reduced in older organisms (13), higher mRNA turnover rates could cause lower transcript levels. Second, the prolonged life span of the transporter proteins in the apical membrane could also contribute to high NaPi IIb amounts at low mRNA levels in adult goats. Third, nutrient transport is restricted to the upper villus region in the older intestine, so that the number of mature enterocytes is decreased, resulting in lower mRNA levels (6, 13). Then an unchanged protein level would indicate a posttranscriptional mechanism to adjust the P_i transport to the P_i demand at this age. Decrease of the NaPi IIb affinity may explain the decrease in transport capacity despite unchanged NaPi IIb protein contents. Decreased NaPi IIa expression in the kidney could correspond to the ontogenetically reduced plasma P_i levels. Because this resulted in lower P_i concentrations in the renal ultrafiltrate, smaller amounts of specific transporters are necessary to maintain ruminants' high renal reabsorption capacity of ~99% (20).

NaPi II mRNA expression in the parotid glands detectable by Northern blot increased from the fourth week of life. However, both function and cellular localization of the parotid NaPi II transporter are still unknown. The developmental change from weaning to fully ruminating goats was characterized by a strong increase in NaPi II mRNA levels in the parotid gland at unchanged salivary P_i concentrations. The amount of salivary P_i secreted into the rumen is correlated to the salivary flow rate. Therefore, the 4- to 5-mo-old goats should have a higher P_i secretion rate due to greater mechanical stimulation in response to intake of solid feed. Furthermore, to maintain the high salivary P_i concentrations of about 30–40 mmol/l, there had to be an increased amount of P_i transporters (NaPi II) expressed in this age group. This also confirms the role of parotid NaPi II for P_i concentrating in saliva.

In conclusion, P homeostasis of growing goats was maintained from the first day of life by an adequate

expression of epithelial Na^+P_i transport capacities in jejunum and kidneys. The special P needs of ruminants due to their rumen ecosystem and their endogenous P_i cycle were satisfied by high intestinal P_i absorption continuously expressed during aging. The high renal P_i reabsorption balanced the high P_i outflow into the rumen when salivary secretion began at weaning. However, newborn and suckling goats were also capable of withholding high amounts of P_i due to the pronounced renal reabsorption capacity. This ability supports the high bone mineralization rate essential in precocial species during ontogenesis.

We thank Prof. W. v. Engelhardt and J. McAlister for critical comments on and correction of the manuscript.

We gratefully acknowledge the financial support of the Deutsche Forschungsgemeinschaft.

REFERENCES

1. Alon U and Chan MD. *Phosphate in Pediatric Health and Disease*. London: CRC, 1993, p. 73–102.
2. Attaix D and Meslin JC. Changes in small intestinal mucosa morphology and cell renewal in suckling, prolonged suckling and weaned lambs. *Am J Physiol Regul Integr Comp Physiol* 261: R811–R818, 1991.
3. Boehnke E, Langner A, and Weissmann F. Zum Phosphat- und Natriumstoffwechsel bei Aufzucht-kälbern. *Zbl Vet Med A* 28: 357–365, 1981.
4. Borowitz SM and Granrud GS. Ontogeny of intestinal phosphate absorption in rabbits. *Am J Physiol Gastrointest Liver Physiol* 262: G847–G853, 1992.
5. Buddington RK. Intestinal nutrient transport during ontogeny of vertebrates. *Am J Physiol Regul Integr Comp Physiol* 263: R503–R509, 1992.
6. Buddington RK. Nutrition and ontogenic development of the intestine. *Can J Physiol Pharmacol* 72: 251–259, 1993.
7. Engelhardt W von, Leonhard-Marek S, Breves G, and Giesecke D. *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction. Proceedings of the 8th International Symposium on Ruminant Physiology*. Stuttgart, Germany: Enke, 1995, p. 153–167.
8. Gimonet V, Bussières L, Medjebeur AA, Gasser B, Lelongt B, and Laborde K. Nephrogenesis and angiotensin II receptor subtypes gene expression in fetal lamb. *Am J Physiol Renal Physiol* 274: F1062–F1069, 1998.
9. Huber K, Walter C, Schröder B, and Breves G. Phosphate transport in the duodenum and jejunum of goats and its adaptation by dietary phosphate and calcium. *Am J Physiol Regul Integr Comp Physiol* 283: R296–R302, 2002.
10. Kruse-Jarres JD. *Klinische Chemie. Spezielle Klinisch-Chemische Analytik*. Stuttgart, Germany: Fischer, 1979, vol. II.
11. Moritz KM and Wintour EM. Functional development of the meso- and metanephros. *Pediatr Nephrol* 13: 171–178, 1999.
12. Mulrone SE, Woda C, and Haramati A. Changes in renal phosphate reabsorption in the aged rat. *Proc Soc Exp Biol Med* 218: 62–67, 1998.
13. Pacha J. Development of intestinal transport function in mammals. *Physiol Rev* 80: 1633–1658, 2000.
14. Schröder B and Breves G. Mechanisms of phosphate uptake into brush-border membrane vesicles from goat jejunum. *J Comp Physiol [B]* 166: 230–240, 1996.
15. Schröder B, Walter C, Breves G, and Huber K. Comparative studies on Na-dependent P_i transport in ovine, caprine and porcine renal cortex. *J Comp Physiol [B]* 170: 387–393, 2000.
16. Sestoft L. Is the relationship between the plasma concentration of inorganic phosphate and the rate of oxygen consumption of significance in regulating energy metabolism in mammals? *Scand J Clin Lab Invest* 39: 191–197, 1979.
17. Taufiq S, Collins JF, and Ghishan FK. Posttranscriptional mechanisms regulate ontogenic changes in rat renal sodium-

- phosphate transporter. *Am J Physiol Regul Integr Comp Physiol* 272: R134–R141, 1997.
18. **Traebert M, Lötscher M, Aschwanden R, Ritthaler T, Biber J, Murer H, and Kaissling B.** Distribution of the sodium/phosphate transporter during postnatal ontogeny of the rat kidney. *J Am Soc Nephrol* 10: 1407–1415, 1999.
 19. **Varyo S, Kemp R, Beechey RB, and Shirazy-Beechey S.** Preparation and characterization of basolateral plasma-membrane vesicles from sheep parotid glands. *Biochem J* 279: 843–848, 1991.
 20. **Widiyono I, Huber K, Failing K, and Breves G.** Renal phosphate excretion in goats. *J Vet Med A* 45: 145–153, 1998.
 21. **Wintour EM, Alcorn D, Albiston A, Boon WC, Butkus A, Earnest L, Moritz K, and Shandley L.** The renin-angiotensin system and the development of the kidney and adrenal in sheep. *Clin Exp Pharmacol Physiol* 25, Suppl: S97–S100, 1998.

