Effect of Pregnancy, Postnatal Growth, and Gender on Renal Sulfate Transport

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Abstract. **Serum sulfate concentrations are increased in infants, young children, and pregnant women, compared with adult values. The objective of this investigation was to examine the influences of age, gender, and pregnancy on renal sulfate transport using guinea pigs as an animal model. Membrane vesicles were isolated from the kidney cortex of male animals at four different ages, from male and female adult animals, and from pregnant and nonpregnant female animals. There were no significant differences in marker enzymes for the brush-border membrane (BBM) or basolateral membrane (BLM) among all groups examined. Uptake was determined by a rapid filtration method and membrane fluidity by measuring the steady-state fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene. The V_{max} values for Na^+/sulfate co-transport in BBM were significantly increased with decreasing age, whereas the K_m for this process was unchanged. The V_{max} and K_m for Na^+/sulfate co-transport in BBM of pregnant animals were significantly higher than the values in the nonpregnant group. Bicarbonate-driven anion exchange of sulfate in BLM was not different among the different age groups. The V_{max} for the bicarbonate/sulfate exchange process in BLM was not different between pregnant and nonpregnant groups; however, the K_m for this process in BLM of pregnant animals was significantly greater than the value in nonpregnant animals. There were no gender-related differences in sulfate transport in BBM or BLM isolated from adult male and female animals. Renal BBM fluidity was increased with decreasing age and in pregnant animals, suggesting that altered membrane fluidity may represent one possible mechanism to explain the increased sodium/sulfate uptake in young and pregnant animals. The higher V_{max} for Na^+/sulfate co-transport in young and pregnant animals suggests that there is an increased density of co-transporter protein or an increase in the rate of movement of the carrier protein (i.e., turnover) once loaded with sodium and sulfate. This increased conservation of inorganic sulfate in young and pregnant guinea pigs may be related to the increased demand for sulfated substrates, such as sulfated glycosaminoglycans, during growth and development.** [P.S.E.B.M. 1999, Vol 221]

Inorganic sulfate is an important physiological anion that is involved in sulfate conjugation of both endogenous and exogenous compounds (1). The plasma or serum concentration of inorganic sulfate is predominantly determined by the kidney, since inorganic sulfate is filtered at the glomerulus and actively reabsorbed in the renal proximal tubules (2, 3). Inorganic sulfate is transported through the brush border membrane (BBM) mainly by sodium-dependent secondarily active transport (4, 5). Although several bivalent anions such as thiosulfate, molybdate, and selenate share this transport process, the sodium/sulfate co-transporter is distinct from the sodium-dependent phosphate, amino acid, or glucose transport proteins (6). The cDNA for the renal sodium/sulfate co-transport protein (NaSi-1) has been identified by Markovich *et al.* (7). Inorganic sulfate is transported out of the proximal tubule cell into the systemic circulation by an exchange mechanism at the basolateral membrane (BLM) (8–10). The sulfate/anion

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transporter (sat-1) of rat kidney has been characterized (11, 12) and is similar to that present on the canalicular membrane of rat liver (12, 13).

Serum sulfate concentrations are elevated in human infants, in children under 3 years of age, and pregnant women (14–16). The urinary excretion of inorganic sulfate in human infants is very low compared with adults (17), suggesting the increased conservation of inorganic sulfate in the young. Renal tubular reabsorption of inorganic sulfate is greater in young (<25 days) than adult (>60 days) guinea pigs (18). The fractional excretion of inorganic sulfate in the urine of third-trimester pregnant women is decreased compared with nonpregnant women, corresponding to a substantial increase in serum sulfate concentration in late pregnancy (19, 20). This increased conservation of sulfate in young and pregnant animals and humans may be related to the increased demand for sulfated substrates during growth and development. Numerous structural components of membranes and tissues are sulfate conjugates: sulfated glycosaminoglycans are components of cartilage and other tissues, whereas cerebroside sulfate is a constituent of the myelin membrane in the brain (21, 22). In tissues, sulfated glycosaminoglycans occur as covalent complexes with a core protein in the form of proteoglycans, and cell differentiation appears to be guided by a tissue-specific composition of sulfated proteoglycans (23). Sulfate conjugation is of key importance in organ development. There is also increasing evidence that sulfated glycosaminoglycans and proteoglycans are involved in modulating cell interactions in developing tissue. For example, heparan sulfate proteoglycans play a role in cell adhesion, neural crest migration, and neurite extension and may be involved in trophic factor regulation (24).

The objective of the present investigation was to characterize the effects of age (<10 days to adult), gender, and pregnancy on renal sulfate transport in a guinea pig animal model by examining transport at both the BBM and BLM in kidneys isolated from the different age groups (neonatal to adult), male and female adult guinea pigs, and pregnant and nonpregnant guinea pigs.

Materials and Methods

Materials. $^{35}SO_4^{2-}$ (as Na_2SO_4, 1050–1600 Ci/mmol) was obtained from New England Nuclear Research Products (DuPont Company, Boston, MA). Biodegradable counting scintillant was obtained from Amersham Corp. (Arlington Heights, IL). Coomassie blue dye reagent concentrate and bovine plasma γ-globulin protein standard were purchased from Bio-Rad (Richmond, CA). 1,6-diphenyl-1,3,5-hexatriene (DPH) was obtained from Molecular Probes (Eugene, OR). Fiske and Subbarow reducer and a commercially available kit to assay maltase activity (kit no. 510-A) were obtained from Sigma Diagnostics (St. Louis, MO).

Renal Membrane Vesicle Preparation and Characterization. Neonatal (<10 days), young (15–20 days and 35–40 days), and adult (90–120 days) male Hartley guinea pigs (Harlan, Indianapolis, IN) were used as kidney donors for the age-dependent studies. The kidneys from pregnant (>50 days in gestation period) and nonpregnant female Hartley guinea pigs (both 90–120 days) (Harlan, Indianapolis, IN) were used for the pregnancy studies. Membrane vesicles were prepared from the kidney cortex using a modification of the procedure of Kinsella *et al.* (25) by a Percoll gradient centrifugation, as previously described (26). The BBM fraction was further purified using $MgCl_2$ precipitation.

Protein concentrations of the homogenate and resultant membrane vesicles were determined by the Coomassie blue binding method (27) using bovine plasma γ-globulin as the protein standard. The purity of the preparations was assessed by determining the activities and enrichment of the membrane marker enzymes, Na^+, K^+-ATPase (28, 29) for the BLM vesicles and maltase (30) for the BBM vesicles.

Vesicle Uptake Procedure. The uptake of inorganic sulfate into BLM and BBM vesicles was determined using a rapid filtration method, as previously described (26). The experiments were begun by adding vesicles to the uptake medium (100 m*M* mannitol, 10 m*M* HEPES, and 100 m*M* NaCl for BBM; 200 m*M* mannitol and 50 m*M* HEPES for BLM; pH 7.5) containing K_2SO_4 and tracer amounts of radiolabeled sulfate (5 μCi/ml) in a 1:10 dilution (to yield a final protein concentration of 0.6 mg/ml). The sample was vortexed, and 100-μl aliquots were removed and filtered at 10 sec. The filters (type HA: pore size, 0.45 μm; Millipore Corp., Bedford, MA) were rinsed with 5 ml of ice-cold stop solution (300 m*M* mannitol, 10 m*M* HEPES for BBM; 200

Table I. Enrichment Ratios of Enzymes in Membrane Vesicles Isolated from Guinea Pigs

	Na^+, K^+-ATPase		Maltase	
	BLM	BBM	BLM	BBM
Adult Male (3–4 mo.)	13 ± 4.0	2.3 ± 0.91	0.82 ± 0.16	8.8 ± 2.4
35–40 days	8.7 ± 2.4	1.9 ± 0.41	0.81 ± 0.14	7.2 ± 2.0
15–20 days	9.5 ± 3.0	1.7 ± 1.0	0.97 ± 0.29	7.2 ± 0.93
Neonate (<10d)	12 ± 2.1	1.4 ± 0.68	0.96 ± 0.21	10 ± 2.1
Adult Female (3–4 mo.)	11 ± 2.1	2.6 ± 0.75	1.1 ± 0.13	12 ± 2.2
Pregnant Female	11 ± 4.9	2.2 ± 0.70	1.0 ± 0.33	11 ± 3.9

Note. Results are presented as mean ± SD (n = 3–7 separate kidney preparations). The enrichment ratio was calculated by the enzyme activity determined in the membrane vesicles divided by that of the homogenate.

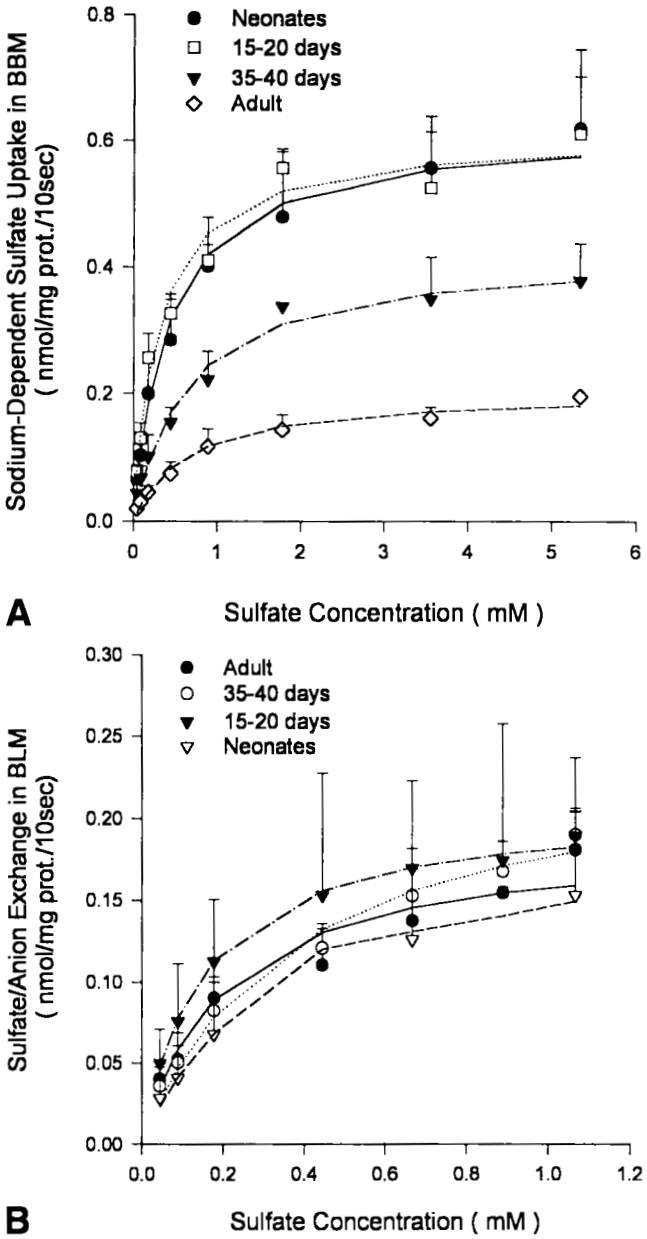

Figure 1. Sodium-dependent uptake of sulfate in BBM and sulfate/anion exchange in BLM vesicles isolated from the kidney cortex of guinea pigs of four different ages. The data were fitted to the Michaelis-Menten equation using nonlinear regression analysis. Each data point is presented as the mean ± SD of four separate kidney preparations, with uptake determined in triplicate for each preparation. (A) The data represent the difference between sulfate uptake rates determined in the presence and absence of sodium in BBM preparations. (B) The data represent the difference between uptake rates determined in the absence and presence of thiosulfate (a competitive inhibitor of sulfate uptake) in BLM preparations. All BLM vesicle preparations contained 50 m*M* $KHCO_3$ in the internal buffer as the driving force for the anion exchanger.

m*M* mannitol, 50 m*M* HEPES for BLM, pH 7.5). Filters were presoaked overnight in the stop solution containing 100 m*M* K_2SO_4 to minimize any binding of inorganic sulfate to the filter itself. Nonspecific filter binding was assessed by filtering radiolabeled uptake media without vesicles. Uptake studies were conducted at 25°C.

Uptake Study Design. The time course of sulfate (200 μ*M*) uptake was determined in BLM and BBM vesicles isolated from guinea pigs of various ages and both pregnant and nonpregnant female animals. Sulfate uptake was examined at 10, 20, 30, 60, 180, and 300 sec and 1 hr. For estimation of V_{max} and K_m for sulfate BBM transport, sulfate uptake into BBM vesicles was determined at 10 sec at various concentrations of sulfate (0.05–6 m*M*) with and without the external presence of 100 m*M* Na^+ (added as NaCl). For studies done in the absence of Na^+, NaCl was replaced with 100 m*M* KCl. The difference between these two values represents the sodium-dependent co-transport process. Sulfate uptake into BLM vesicles, which were preincubated with 50 m*M* $KHCO_3$, was evaluated over the concentration range of 0.05 m*M*–1.2 m*M* sulfate in the presence and absence of 20 m*M* thiosulfate (as the potassium salt), an inhibitor of the facilitated transport of sulfate. The difference between the uptake values at the same sulfate concentrations with and without thiosulfate represents the anion-exchange process, which is driven by intravescular bicarbonate. Previous studies in the laboratory have demonstrated that the 10-sec uptake value provides an estimate of the linear uptake of sulfate in both BBM and BLM (26). Each study was performed using between three and five separately isolated kidney cortex preparations, with uptake values determined in triplicate in each kidney preparation. A single kidney preparation involved the use of one adult animal, but larger numbers of young animals were used to obtain the same amount of kidney cortex.

Evaluation of Membrane Fluidity (Motional Order). The fluidity of BBM and BLM obtained from kidneys of guinea pigs at different ages and of pregnant and nonpregnant animals was evaluated by examining the fluorescence polarization of DPH. Membrane vesicles were diluted with a phosphate-buffered saline solution (PBS, pH 7.4) to a protein concentration of 0.1 mg/ml. Two μl of 2 m*M* DPH in tetrahydrofuran was added to 2 ml of membrane vesicles. Fluorescence polarization measurements were performed using an SLM Aminco (SLM Aminco, Urbana, IL) 8000 spectrofluorometer with film polarizers (FP110) at a temperature varying between 20°C and 50°C with the excitation wavelength of 355 nm and the emission wavelength of 430 nm (31). Corrections for light scattering were applied by conducting appropriate control experiments without added probe (32).

Data Analysis. Data representing uptake of inorganic sulfate over a range of concentration, evaluated at 10 sec, were fitted to the Michaelis-Menten equation by nonlinear regression analysis (PCNONLIN, Statistical Consultants Inc., Lexington, KY) to obtain estimates of the K_m and V_{max} values for the uptake processes. Data were fitted with a weighting factor, 1/variance (26). Initial parameter estimates were obtained from Lineweaver-Burk plots.

Statistical Analysis. All results were expressed as the mean ± SD. For age-dependent studies, differences in the estimates of K_m and V_{max} for sulfate renal transport were

compared by ANOVA followed by a Tukey's test among groups. For the pregnancy and gender studies, the data were compared by an unpaired Student's t test between groups. The differences were considered to be statistically significant when $P < 0.05$.

Results

Renal Membrane Vesicle Characterization. The protein concentrations from BBM and BLM vesicles were not different when isolated from neonatal, young, or adult guinea pigs. Nor were there differences in protein content in vesicles isolated from male and female adult animals or between pregnant and nonpregnant females. The BBM and BLM vesicle preparations were characterized by examining the enrichment of enzymes present in the BBM and BLM, compared with that in the homogenate. The BBM enzyme enrichment for maltase, a marker for BBM, was 7.2 ~ 10, whereas the BLM fractions had enrichment ratios for Na^+, K^+-ATPase, a marker enzyme for BLM, of 8.7 ~ 13 among the various age groups. These values were not statistically different among groups. No significant difference was found in enrichment ratios of maltase or Na^+, K^+-ATPase between pregnant and nonpregnant animals or male and female adult animals (Table I).

Time Course of Sulfate Uptake into BLM and BBM Vesicles. Sulfate uptake into BBM and BLM vesicles, examined at various times, exhibited an initial overshoot during the first 5 min of incubation, indicating an intravesicular accumulation of sulfate above the equilibrium concentration. The uptake of sulfate into BLM and BBM vesicles reached equilibrium after 60 min (data not shown).

Concentration-Dependent Sulfate Uptake into BLM and BBM Vesicles. In all studies, sulfate uptake into BBM vesicles was greater in the presence of Na^+ compared with that in the absence of Na^+, demonstrating the sodium dependence of the transport process (data not shown). Sodium-dependent sulfate BBM uptake was significantly decreased with increasing age (Fig. 1A). The values for V_{max} and K_m determined from PCNONLIN fits of the Michaelis-Menten equation are presented in Table II. The V_{max} estimate for the Na^+/sulfate co-transport in BBM vesicles was significantly higher in younger animals compared with adults (3–4 months). However, the value for V_{max} between neonates (<10 days) and 15–20-day-old guinea pigs was not statistically different. There was no significant change in the K_m for sulfate BBM uptake.

Sulfate uptake into BLM vesicles was decreased in the presence of 20 mM thiosulfate, a competitive inhibitor for the BLM sulfate anion exchanger (data not shown). As previously characterized by Darling *et al.* (26), the difference between sulfate uptake rates determined in the absence and presence of 20 mM thiosulfate represents the bicarbonate-driven sulfate anion exchange process (Fig. 1B). There were no significant differences in the V_{max} and K_m estimates for BLM anion exchange among the various ages (Table II).

Sodium/sulfate co-transport was higher in pregnant guinea pigs than in nonpregnant animals (Fig. 2A). The V_{max} and K_m estimates for sodium-dependent sulfate transport were significantly increased in pregnant animals compared with nonpregnant guinea pigs (Table III). Bicarbonate-driven sulfate anion exchange was observed in the BLM vesicles (Fig. 2B). The V_{max} for bicarbonate-dependent anion exchange of sulfate in BLM was not different between groups, whereas the K_m estimate for this process in BLM of pregnant guinea pigs was significantly greater than the value in nonpregnant animals (Table III).

There were no significant differences in sodium/sulfate uptake in BBM or sulfate/anion exchange in BLM between male and female adult guinea pigs (Table IV; Figs. 3A and 3B).

Membrane Fluidity. The fluorescence polarization of DPH as a function of temperature was determined in BBM and BLM prepared from 18- and 36-day-old and adult male guinea pigs, and in female pregnant and nonpregnant guinea pigs. The steady-state fluorescence polarization of DPH represents membrane motional order in the hydrophobic core of the lipid bilayer and is inversely related to membrane fluidity (31, 32). There was a significant increase in fluidity in BBM (Figs. 4A and 4B) and in BLM (data not

Table II. Transport Parameter Estimates for Sodium-Dependent Sulfate Uptake in BBM and Sulfate/Anion Exchange in BLM Vesicles Isolated from the Kidney Cortex of Guinea Pigs of Four Different Ages

Age	BBM		BLM	
	V_{max} (nmol/mg prot/10sec)	K_m (μM)	V_{max} (nmol/mg prot/10sec)	K_m (μM)
Adult Male (3–4 mo.)	0.19 ± 0.03[b,d,e]	540 ± 85	0.20 ± 0.02	220 ± 77
35–40 days	0.38 ± 0.07[a,c,e]	473 ± 250	0.23 ± 0.05	210 ± 56
15–20 days	0.62 ± 0.08[c,d]	370 ± 96	0.21 ± 0.05	170 ± 51
Neonates (<10 days)	0.63 ± 0.07[a,b]	380 ± 120	0.21 ± 0.07	320 ± 140

Note. Values are presented as mean ± SD of four separate kidney preparations, with the uptake values determined in triplicate in each preparation. The estimates for V_{max} describing the sodium-dependent uptake in BBM were significantly different among groups except those for neonates and 15–20-day-old guinea pigs. V_{max} values with the same superscript were compared and found to be statistically different: (a, b, c, d,) $P < 0.001$; (e) $P < 0.05$. The K_m values for sodium-dependent uptake in BBM and the K_m and the V_{max} values for sulfate/anion exchange in BLM were not statistically different among the age groups.

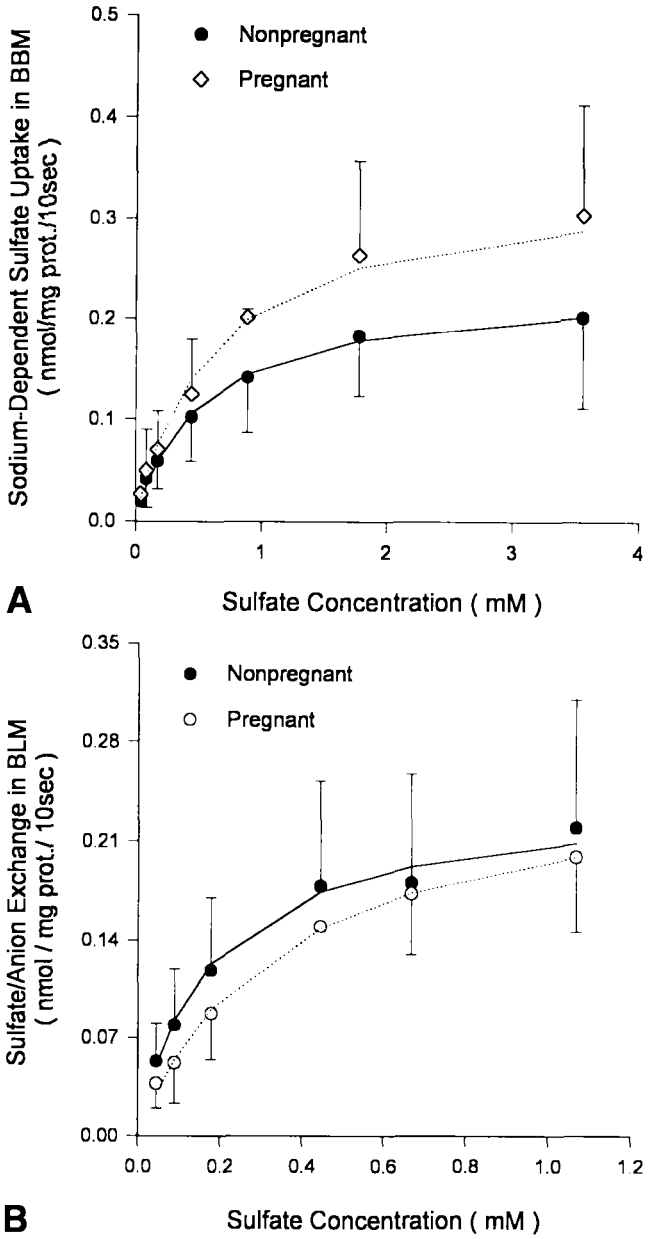

Figure 2. Sodium-dependent uptake of sulfate in BBM and sulfate/anion exchange in BLM vesicles isolated from the kidney cortex of pregnant and nonpregnant guinea pigs. See the Figure 1 legend. Each data point is presented as the mean ± SD of three to four separate kidney preparations; for each preparation, the uptake values were determined in triplicate. (A) Sodium-dependent sulfate uptake in BBM. (B) Sulfate/anion exchange in BLM.

shown) isolated from young and pregnant guinea pig kidney cortex.

Discussion

The present investigation studied age-, gender-, and pregnancy-induced alterations in sulfate renal transport using guinea pigs as an animal model. Nephrogenesis in guinea pigs is complete within the gestation period, like in humans, but unlike in other animals such as rat, rabbit, and dog in which nephron formation continues after birth (33, 34). Similar enrichment ratios of membrane marker enzymes, Na^+, K^+-ATPase for BLM and maltase for BBM demonstrated that the membrane vesicle preparations were not different among various ages of guinea pigs and between pregnant and nonpregnant animals.

Sodium-dependent sulfate co-transport in BBM vesicles and sulfate/anion exchange in BLM vesicles were present in young guinea pigs. Sodium-driven sulfate uptake into BBM vesicles was significantly higher in the younger age groups. The age-related change in sulfate renal uptake into BBM vesicles was primarily due to differences in V_{max} because the K_m values were similar among the four different groups. This finding is consistent with the report of Pena *et al.* (18) that showed a higher V_{max} but no change in K_m for sodium/sulfate co-transport in BBM vesicles isolated from the kidneys of young (<25 days) guinea pigs compared with adult (>60 days) animals. We additionally observed no alteration in sulfate/anion exchange in BLM vesicles. Sodium/phosphate transport in the kidney is also characterized by a higher V_{max} in BBM isolated from neonatal and young animals compared with adult animals (35, 36). However, the V_{max} for sodium/glucose transport in kidney cortex BBM is similar in neonatal and adult animals (35).

Although both the V_{max} and K_m estimates for sodium/sulfate co-transport in BBM vesicles isolated from pregnant guinea pigs were significantly higher than the values in nonpregnant animals, sodium-dependent sulfate BBM uptake in pregnant animals was greater than in nonpregnant animals over a wide range of sulfate concentrations examined. This result corresponds to the report of Cole *et al.* (20) that fractional reabsorption of sulfate was increased in women in the third trimester of pregnancy. In BLM vesicles, bicarbonate-driven sulfate uptake was not different between groups, although the K_m for the sulfate/anion exchange process in pregnant guinea pigs was significantly greater in BLM isolated from pregnant animals, with no significant difference in the V_{max}. Our work reported here represents the first examination of renal sulfate transport during pregnancy.

Another finding in this study was that there were no gender differences in the V_{max} and K_m estimates for sodium-dependent sulfate transport in BBM vesicles and for bicarbonate-driven sulfate exchange in BLM vesicles isolated from male and female adult guinea pigs. Although there is a paucity of information in the literature concerning the effect of gender on transport processes, a few reports have noted gender-related differences in the renal transport of anions and cations (37–40). Renal uptake of p-aminohippurate, tetramethylammonium, and amantadine are significantly greater in male rats than in females (37–39). In human studies, the postsecretory reabsorption of urate is significantly increased in the kidneys of adult men compared with adult women (40). Tallgren (19) previously reported that there is no significant difference in serum sul-

Table III. Transport Parameter Estimates for Sodium-Dependent Sulfate Uptake in BBM and Sulfate/Anion Exchange in BLM Vesicles Isolated from the Kidney Cortex of Pregnant and Nonpregnant Guinea Pigs

Age	BBM		BLM	
	V_{max} (nmol/mg prot/10sec)	K_m (μM)	V_{max} (nmol/mg prot/10sec)	K_m (μM)
Nonpregnant	0.22 ± 0.06[a]	390 ± 140[a]	0.23 ± 0.08	140 ± 24[b]
Pregnant	0.40 ± 0.06	700 ± 99	0.32 ± 0.15	540 ± 81[b]

Note. Values are reported as mean ± SD of three to four separate kidney preparations, with the uptake values determined in triplicate for each preparation. The estimates for V_{max} and K_m for sodium-dependent uptake in BBM and the K_m for sulfate/anion exchange in BLM in pregnant guinea pigs were significantly higher than those in the nonpregnant group. (a) $P < 0.05$; (b) $P < 0.001$.

fate concentrations between men and women. The influence of gender on sulfate renal transport has not been previously examined.

The observed changes in renal sulfate transport in neonatal, young, and pregnant animals may be due to a number of factors, including (1) alterations in the sodium gradient, which represents the driving force for BBM uptake; (2) effects of hormones such as growth hormone or estrogen/progesterone on NaSi-1 protein expression; and (3) effects of altered membrane lipid composition and fluidity. It is unlikely that the changes in sulfate transport during growth, development, and pregnancy are due to the alterations in the electrical driving force or in the rate of dissipation of the sodium gradient, since these have been reported to be unchanged (18, 41). Additionally, there were no differences in Na^+ K^+-ATPase activity among groups in the present investigation.

Little is known regarding the role of hormones involved in growth and development, such as growth hormone (GH) and insulin-like growth factor-1 (IGF-1), or 17β-estradiol and progesterone, which are elevated in pregnancy, on the renal reabsorption of sulfate. Renal sulfate reabsorption was reported to be increased in dogs treated with GH (42), and in a patient with acromegaly and gigantism (43). This effect of GH may be mediated through IGF-1 since IGF-1 itself increases the tubular reabsorption of inorganic phosphate and stimulates sodium/phosphate transport (44, 45). Tallgren (19) demonstrated changes in serum sulfate concentrations during the menstrual cycle, suggesting that progesterone may modulate sulfate homeostasis. Menopause results in lower serum sulfate concentrations, and the renal reabsorption of sulfate is lower in postmenopausal women compared with premenopausal women (46). We have investigated the effect of GH, IGF-1, 17β-estradiol, and progesterone on sodium/sulfate uptake in Madin-Darby Canine Kidney (MDCK) cells that have been stably transfected with NaSi-1 (47). The results demonstrated increased sulfate uptake (48), suggesting the possibility that these hormones may influence sodium/sulfate co-transport.

Third, it is possible that changes in membrane lipid composition or fluidity may affect sulfate transport. In studies examining the steady-state fluorescence polarization of DPH, a probe that has been extensively used to investigate the fluidity of the hydrophobic core of the membrane lipid bilayer (31, 32), we found an increased fluidity in both BBM and BLM isolated from neonatal, young, and pregnant animals. These results are consistent with the report of Medow *et al.* (49) that membrane fluidity was increased with decreasing age in rat kidney BBM preparations. Membrane motional order has been shown to affect numerous membrane functions including transport systems, activities of enzymes, and permeability (50). A change in membrane fluidity does not affect all transport proteins in the same manner; for example, with increased BBM fluidity, there is decreased sodium/glucose transport but increased sodium/phosphate transport (50). We have found in additional studies that an increase in fluidity of BBM of MDCK cells that have been stably transfected with NaSi-1 cDNA, resulting from benzyl alcohol administration, increased the V_{max} of sodium-dependent sulfate transport, whereas a decrease in BBM fluidity of cells produced by preincubation with cholesterol resulted in a decrease of the V_{max} for this process with no significant alteration in K_m (unpublished results). We have also found that benzyl alcohol, at 5 and 20 mM concentrations, significantly stimulated sodium-dependent sulfate uptake at 10 sec (linear uptake) in BBM vesicles

Table IV. Transport Parameter Estimates for Sodium-Dependent Sulfate Uptake in BBM and Sulfate/Anion Exchange in BLM Vesicles Isolated from the Kidney Cortex of Male and Female Guinea Pigs

Gender	BBM		BLM	
	V_{max} (nmol/mg prot/10sec)	K_m (μM)	V_{max} (nmol/mg prot/10sec)	K_m (μM)
Male	0.19 ± 0.03	540 ± 85	0.20 ± 0.02	220 ± 77
Female	0.22 ± 0.06	390 ± 140	0.23 ± 0.08	140 ± 24

Note. Values are reported as mean ± SD of four to five separate kidney preparations, with the uptake values determined in triplicate for each preparation. There were no significant differences in kinetic parameters between the male and female guinea pigs.

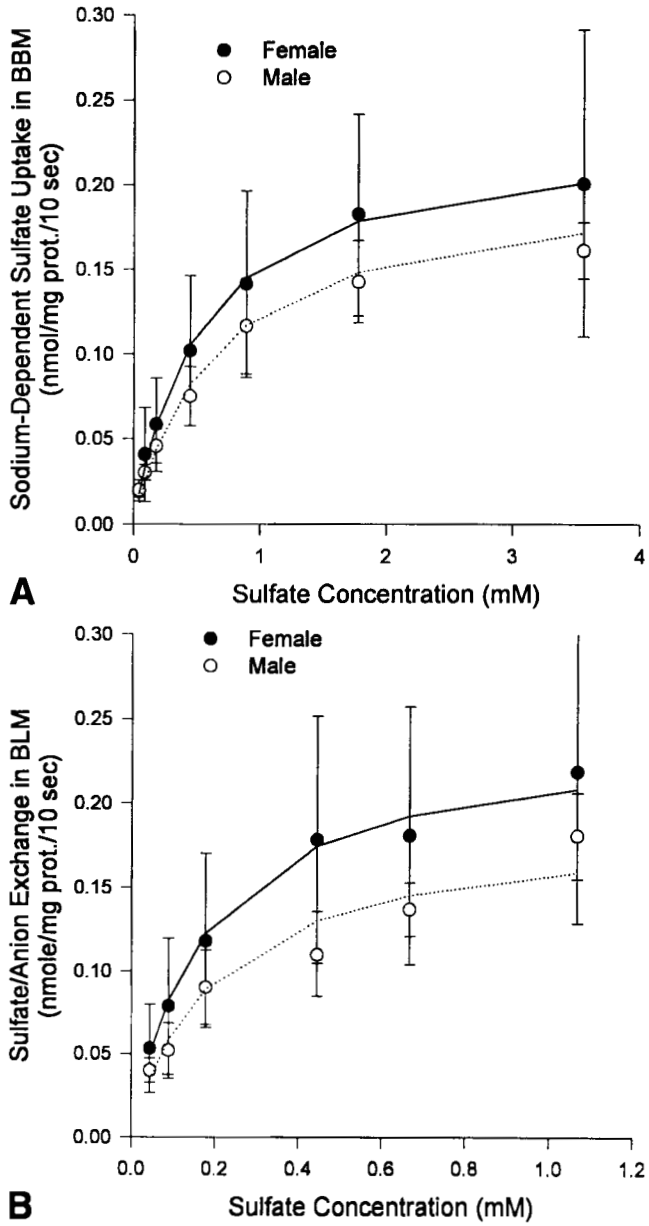

Figure 3. Sodium-dependent uptake of sulfate in BBM and sulfate/anion exchange in BLM vesicles isolated from the kidney cortex of male and female adult guinea pigs. See the Figure 1 legend. Each data point is presented as the mean ± SD of four to five separate kidney preparations; for each preparation, the uptake values were determined in triplicate. (A) Sodium-dependent sulfate uptake in BBM. (B) Sulfate/anion exchange in BLM.

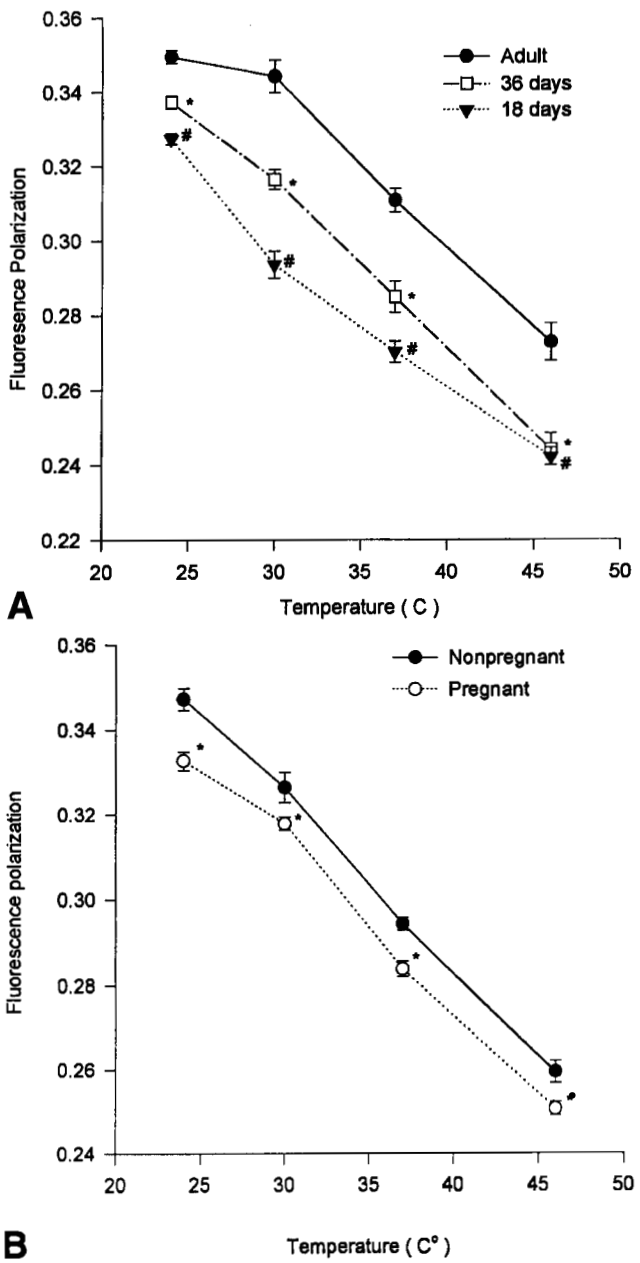

Figure 4. Fluorescence polarization of DPH in BBM isolated from guinea pigs as a function of temperature. The fluorescence polarization of DPH as a function of temperature was determined in (A) BBM vesicles prepared from 18-day and 36-day-old and adult male guinea pigs. Four measurements were taken per point for each preparation. Each data point represents the mean ± SD $^{*,\#}P < 0.001$ compared with adult. (B) BBM vesicles isolated from pregnant and nonpregnant guinea pigs. Each data point is presented as the mean ± SD from four measurements. $^{*}P < 0.005$ compared with pregnant female guinea pigs.

isolated from rat kidney cortex (unpublished results). The changes in membrane fluidity may reflect specific differences in membrane lipid composition present in pregnant or young animals, for example, changes in cholesterol content and those specific lipid alterations may modulate cell function by a number of mechanisms including transcriptional regulation (51, 52). Levi *et al.* (53) demonstrated that the decrease in BBM sodium/phosphate transport in old rats is associated with an age-dependent decrease in renal BBM cholesterol content, and that alterations in BBM cholesterol can produce decreases in BBM sodium/phosphate co-transport. It has been suggested that the rigid lipid environment in the BBM of aged animals may hinder the conformational changes important for the sodium/phosphate transport process (54). Therefore, the increase in BBM fluidity in young and pregnant animals may be responsible, at least in part, for the observed changes in sodium/sulfate co-transport.

In conclusion, there were no gender-related differences

in sodium/sulfate uptake in BBM or sulfate/anion exchange in BLM in adult guinea pigs. Significant age-dependent and pregnancy-induced alterations occur in renal sodium/sulfate uptake in BBM vesicles, with neonatal, young, and pregnant animals exhibiting an increased V_{max} for sodium/sulfate co-transport.

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