leading edge of descending red cells. Stomatocyte predilection at acid pH is modified as a thickening of the advancing edge and a thinning of the trailing edge. Red cell deformability as measured by ektacytometry is controlled by the relationship of the surface area to the volume of the cell, the internal viscosity of hemoglobin and the intrinsic rigidity of the red cell membrane[10]. In this technique, using a phosphate buffer solution at pH 7.4, factors that determine the elongation of the trailing edge include the relationship of surface area to volume and intrinsic characteristics of the membrane itself, because the elongated end portion of the cell becomes squeezed free of cell contents. The photographs suggest that it is more likely that the echinocytes and possibly even the stomatocytes have a smaller surface area than the normal red cells, and it is of interest that the echinocyte can lose some of its spicules when force is exerted on the inside of the membrane. This simple and inexpensive technique using a capillary tube will be useful for the investigation of rheological membrane properties.

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Increased urinary excretion of cyclic nucleotides in X-linked hypophosphatemic *(Hyp)* mice

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Summary. Hyp mice, a model for human X-linked hypophosphatemia, had elevated urinary cyclic AMP, cyclic GMP, and magnesium excretion compared to normal mice. The data suggest a renal origin of the urinary cyclic nucleotides. No significant differences in plasma cyclic AMP and cyclic GMP were observed between genotypes.

Introduction. A mutant, X-linked dominant gene *(Hyp)* has been reported in mice[2] which results in a phenotype similar to that in human X-linked hypophosphatemia (XLH), a form of vitamin D-resistant rickets[3]. The disease in mice, as in humans, is characterized by increased renal excretion of phosphate (P), low plasma P (hypophosphatemia) and osteomalacia[2-4]. The etiology of XLH is unknown. Elevated urinary cyclic AMP (UcAMP) has recently been reported in *Hyp* mice[5-7]. But in those studies plasma cAMP was not measured, and consequently, it was not possible to determine whether the high UcAMP was simply due to elevated filtered load. Our study was undertaken to determine if the elevated UcAMP in *Hyp* mice was of renal origin. In addition, urinary excretion of cyclic GMP (UcGMP) - a parameter previously unexamined in XLH - was measured because cGMP, like cAMP[8,9], appears to be involved in the regulation of calcium and phosphate[10,11]. Finally, renal excretion of electrolytes was measured in order to confirm that the *Hyp* mice used in these studies had elevated excretion of P, and to determine if the renal excretion of any other electrolyte was also elevated.

Materials and methods. The breeding and maintenance of normal and *Hyp* C57BL/6J mice has previously been described[4]. The mean age of intact normal and *Hyp* mice used in this study was 13.5 ± 0.1 weeks, mean $\pm$ SE. Immediately prior to blood collection mice were held over a beaker and the spontaneously voided bladder urine was collected. Blood was then collected into heparinized microfuge tubes by cutting the carotid artery. Cyclic nucleotides were measured in urine and plasma samples by the double antibody radioimmunoassay (RIA) developed according to the method of Steiner et al.[12] using reagents commercially available from New England Nuclear (Boston, Mass; RIA kits NEX-132 and NEX-133). The validity of the RIA was verified in our laboratory by standard procedures[13]. Treatment of samples with phosphodiesterase (PDE) demonstrated the absence of immunoreactive substances (other than cyclic nucleotides) in plasma and urine. We have found that plasma PDE activity causes degradation of plasma cyclic nucleotides resulting in low plasma cyclic nucleotide values[14,15]. This problem can be minimized by rapid processing of samples - in our laboratory PDE was inactivated within 5 min after the onset of blood drawing by adding plasma to TCA. Based on degradation curves determined in our laboratory (not shown), the data shown herein represent at least 90% of the actual plasma cyclic nucleotide levels. Ether anesthesia did not influence plasma cyclic nucleotide levels. Procedures for measuring electrolytes have previously been described[4]. Plasma and urinary creatinine were analyzed using a modification of the kinetic method of Lustgarten and Wenk[16]. The fractional excretion (clearance ratio) of cyclic nucleotides was calculated as follows: fractional excretion (FE) = (urine/plasma concentration of cyclic nucleotide) $\div$ (urine/plasma concentration of creatinine) $\times$ 100.

Results. No significant difference (NSD) in plasma cyclic nucleotides was seen between *Hyp* and normal mice. Compared to normal miee, *Hyp* mice displayed significantly elevated UcAMP and UcGMP regardless of whether data were expressed as a fractional excretion or as nmole cyclic nucleotide/mg urinary creatinine. No significant genotype differences were observed for renal excretion of Ca (table) or Na and K (data not shown). The high FE-P, characteristic of XLH, was expressed to the same degree in both heterozygous *Hyp* females and hemizygous *Hyp* males ($40 \pm 4\%$ in *Hyp* females, $n = 14$, vs $21 \pm 2\%$ in normal females, $n = 17$; $37 \pm 4\%$ in *Hyp* males, $n = 16$, vs $21 \pm 3\%$ in normal males, $n = 17$, $p < 0.001$). Excretion of Mg was significantly greater in pooled *Hyp* mice compared to normals, confirming our preliminary report[15]. Elevated urinary Mg excretion in *Hyp* mice has also been observed by others[7], and may be related to the altered Mg metabolism previously reported[4].

Plasma and urine parameters in intact mice

Parameter	Male Normal	*Hyp*	Female Normal	*Hyp*	ANOVA Genotype	Sex
Plasma cAMP	149± 9 (15)	144± 8 (16)	150± 10 (7)	131± 12 (7)	NSD	NSD
Plasma cGMP	54± 4 (8)	53± 3 (10)	61± 3 (8)	55± 8 (10)	NSD	NSD
FE-cAMP	215± 16 (12)	288± 21 (14)[1]	179± 11 (7)	247± 14 (7)[1]	$p<0.01$	NSD
FE-cGMP	205± 10 (7)	325± 28 (6)[1]	218± 19 (7)	234± 8 (6)[2]	$p<0.001$	NSD
nmole cAMP/mg creatinine	37± 1.7 (34)	47± 1.6 (32)[1]	34± 1.7 (33)	49± 2.4 (31)[1]	$p<0.001$	NSD
nmole cGMP/mg creatinine	9.5± 0.8 (25)	16± 1.0 (25)[1]	14± 0.7 (23)[2]	15± 0.9 (25)	$p<0.001$	$p<0.05$
µmole Ca/mg creatinine	1.2± 0.1 (22)	1.6± 0.2 (14)	2.8± 0.2 (21)[2]	3.5± 0.5 (16)[2]	NSD	$p<0.001$
µmole Mg/mg creatinine	57± 3.4 (41)	64± 3.0 (26)	66± 5.1 (21)	80± 5.4 (22)[1,2]	$p<0.02$	$p<0.01$
Creatinine: Plasma	61.1± 1.0 (30)	59.1± 1.1 (33)	59.5± 1.6 (20)	58.1± 2.6 (21)	NSD	NSD
Urine (cAMP data)	607± 40 (34)	644± 41 (32)	536± 45 (28)	488± 32 (26)[2]	NSD	$p<0.01$
Urine (cGMP data)	532± 33 (25)	589± 42 (25)	480± 30 (23)	494± 28 (25)	NSD	$p<0.05$

Values are mean± SE. Samples size is in parentheses. Units for plasma cyclic nucleotides are pmoles/ml plasma. FE = fractional excretion × 100. Units for plasma creatinine are µM; units for urine creatinine are mg/l urine. ANOVA = 2-way analysis of variance which compared data by genotype (pooled *Hyp* vs pooled normal mice) and by sex (pooled males vs pooled females). Superscript 1 indicates significant difference ($p<0.05$) between *Hyp* and normal mice of the same sex; superscript 2 indicates significant difference ($p<0.05$) between males and females of the same genotype (comparisons made by Duncan's multiple range test).

A significant effect of sex was observed with the urinary cGMP, Ca and Mg data, in which pooled females had greater values than pooled males. This apparent sex effect for UcGMP and urinary Mg can be attributed to the lower urinary creatinine values observed in normal and *Hyp* female mice, since no effect of sex was evident when the urinary concentrations of cGMP and Mg were analyzed by 2-way analysis of variance (data not shown). In contrast, the effect of sex in the urinary Ca data is real, as the urinary Ca concentrations (µmole/l urine) were significantly higher ($P < 0.001$) in pooled females (1.77± 0.16 µmole/l, n = 48) compared to males (0.87± 0.08, n = 50). Low urinary creatinine levels in females compared to males have also been observed in humans[17]. This sex difference may be a manifestation of lower muscle mass in females[17,18], since female mice have lower body weights than males. Alternatively, renal handling of creatinine may be different between sexes, attributable to a variety of factors such as circulating levels of testosterone[19].

Discussion. The FE-cAMP and FE-cGMP were both greater than 100%, the theoretical FE-inulin. cAMP/creatinine clearance ratios exceeding 100% have also been observed in humans[17]. Consequently, UcAMP and UcGMP in normal and *Hyp* mice originates from either the peritubular capillaries or from the renal tubular cells themselves (or both), in addition to glomerular filtration. However, since NSD existed in plasma cyclic nucleotides between genotypes, the elevated UcAMP and UcGMP in *Hyp* mice cannot simply be attributed to elevated filtered load.

Interestingly, the plasma and urine cyclic nucleotide values in C57BL/6J normal and *Hyp* mice were 5–10 times greater than values generally observed in humans or rats[17,20]. We suspect this represents a species difference since others have also reported high plasma cAMP[21] and urinary cAMP[5–7] levels in mice. High values could conceivably be attributable to nonspecific binding or poor assay sensitivity and specificity resulting in cross-reactions with other nucleotides. However, the acetylation of samples and standards, and the use of phosphodiesterase blanks, theoretically diminishes or eliminates these potential problems (see materials and methods section).

We have recently found that intact *Hyp* mice are characterized by secondary hyperparathyroidism[22]. However, this condition can only partially explain the elevated FE-P and UcAMP, since FE-P and UcAMP remain significantly greater in thyroparathyroidectomized *Hyp* mice compared to thyroparathyroidectomized normal mice[23].

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