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A candidate mouse model for Hartnup Disorder deficient in neutral amino acid transport

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Abstract. The mutant mouse strain HPH2 (hyperphenylalaninemia) was isolated after N-ethyl-N-nitrosourea (ENU) mutagenesis on the basis of delayed plasma clearance of an injected load of phenylalanine. Animals homozygous for the recessive *hph2* mutation excrete elevated concentrations of many of the neutral amino acids in the urine, while plasma concentrations of these amino acids are normal. In contrast, mutant homozygotes excrete normal levels of glucose and phosphorus. These data suggest an amino acid transport defect in the mutant, confirmed in a small reduction in normalized values of ^{14}C-labeled glutamine uptake by kidney cortex brush border membrane vesicles (BBMV). The hyperaminoaciduria pattern is very similar to that of Hartnup Disorder, a human amino acid transport defect. A subset of Hartnup Disorder cases also show niacin deficiency symptoms, which are thought to be multifactorially determined. Similarly, the HPH2 mouse exhibits a niacin-reversible syndrome that is modified by diet and by genetic background. Thus, HPH2 provides a candidate mouse model for the study of Hartnup Disorder, an amino acid transport deficiency and a multifactorial disease in the human.

Introduction

Hartnup Disorder is an autosomal recessive human condition identified by a characteristic hyperaminoaciduria (Levy 1995; Baron et al. 1956) that occurs at a frequency of approximately 1 in 40,000 in urine amino acid screens (Wilcken et al. 1977; Lemieux et al. 1988; Levy 1995). Although kidney amino acid transport has not been directly assessed, plasma amino acid levels are usually normal, indicating a transport defect in the kidney tubule. The pattern of hyperaminoaciduria sets Hartnup Disorder apart from other known amino acid transport deficiencies. For example, in contrast to diseases such as cysinuria (Segal and Thier 1995), lysinuric protein intolerance (Simell 1995), or familial aminoglycinuria (Chesney 1995), in which only a few structurally similar amino acids are affected, Hartnup Disorder affects the transport of a large number of neutral amino acids.

The diagnostic hyperaminoaciduria is the only consistent element of a complex and variable phenotype (Baron et al. 1956; Scriver 1965; reviewed by Levy 1995). Fecal amino acid levels are elevated in some (Scriver 1965; Pomeroy et al. 1968), but not all (Shih et al. 1971) patients. Further, indole compounds, secondary metabolites of excess tryptophan (Shaw et al. 1960; Asatoor et al. 1963), are found in the urine, plasma, and feces of some patients. Normally, oral amino acid challenge results in a transient increase in plasma levels for that amino acid. However, in Hartnup Disorder patients, this effect is much reduced and may be delayed (Scriver 1965; Halvorsen et al. 1969; Shih et al. 1971; Tarlow et al. 1972). These observations indirectly indicate an intestinal amino acid transport defect similar to that in the kidney, at least in some patients. Using a direct measure of transport, several investigators have reported deficient amino acid uptake by the brush border of intestinal mucosa biopsies from Hartnup patients (Shih et al. 1971; Tarlow et al. 1972). However, transport by leukocytes (Tada et al. 1966) and fibroblasts (Groth and Rosenberg, 1972) is not affected.

Curiously, the original Hartnup Disorder proband was brought to the attention of a physician not by urine amino acid screening nor by amino acid transport deficiency, but by an apparent case of pellagra, a disease of niacin deficiency (Baron et al. 1956). The symptoms included a photosensitive skin rash, ataxia, and ''psychotic behavior.'' Symptoms occurred despite apparent normal dietary niacin intake, yet disappeared following niacin therapy. Subsequent examination of urinary amino acids revealed the striking hyperaminoaciduria. Other patients presenting with similar niacin-deficiency symptoms (despite apparently normal dietary niacin intake) were found to have the same amino acid profile in the urine (Scriver 1965; Nielsen et al. 1966; Pomeroy et al. 1968; Seakins, 1977). Thus, Hartnup Disorder was considered a rare disease characterized by hyperaminoaciduria and niacin deficiency symptoms. With the advent of prospective urine amino acid screening, however, it has become clear that the Hartnup amino acid transport defect is usually benign (Wilcken et al. 1977; Scriver et al. 1987; Lemieux et al. 1988), and the majority of those identified by urine amino acid screening never display the symptoms of pellagra.

Symptomatic and asymptomatic cases of Hartnup Disorder can occur in siblings with similar hyperaminoacidurias (Baron et al. 1956; Nielsen et al. 1966; Pomeroy et al. 1968). Scriver (1988) and Scriver and colleagues (1987) have proposed that Hartnup Disorder is a single gene disorder, but a multigene disease. Thus, a single recessive mutation in amino acid transport is responsible for the hyperaminoaciduria but is not sufficient to cause a disease state. Therefore, multiple loci determine whether an individual is sensitive to the environmental stimuli that trigger symptoms.

A study of Hartnup Disorder involves understanding the physiology and genetics of amino acid transport as well as the genetic and environmental determinants resulting in the transition from a healthy to a pathologic state. An animal model would allow such investigations under controlled conditions of environment and genetic background. Among several recessive mutations in the mouse, isolated on the basis of delayed clearance of a phenylalanine challenge (McDonald and Bode 1988; McDonald et al. 1990; Shedlovsky et al. 1993), one is defective in amino acid transport and provides a possible model for Hartnup Disorder. Genetic analysis of this mutant, HPH2, is described in the accompanying

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paper (Symula et al. 1996). Here we describe the mutant phenotype and its similarity to Hartnup Disorder.

Materials and methods

Animals. The HPH2 mutant was isolated as described in McDonald and associates (1990). Mice were bred and maintained by standard methods of mouse husbandry (Les, 1966). All mice were bred and maintained on Purina 5001 feed containing niacin at 95 ppm (Purina Mills, Richmond, Ind.) or, where noted, Harlan Teklad Breeder Blox containing niacin at 67 ppm (Harlan Teklad, Madison, Wis.). HPH2 mice were congenic on BTBR (N = 13). The (SWR × BTBR) *hph2*/ + F_1 × BTBR-*hph2*/*hph2* cross is described elsewhere (Symula et al. 1996).

Amino acid profiles. Urine was collected by massaging the bladder contents in a petri dish. Samples were pipetted into 1.5-ml microfuge tubes (Brinkmann, Westbury, N.Y.) and stored at −20°C. Blood (200–300 μl), collected from the retro-orbital sinus, was centrifuged in 1.5-ml microfuge tubes at approximately 7000 *g* for 1 min. Plasma was drawn off and stored at −20°C. These samples were collected at standardized times of the day, between 3 and 6 P.M. Amino acids were quantitated with a Beckman 6300 Automated Amino Acid Analyzer by the method of Slocum and Cummings (1991).

Glucose and phosphorus. Glucose was determined by procedure 16-UV (Sigma Diagnostics, St. Louis, Mo.). Samples were collected at approximately 3 P.M. Phosphorus determinations were performed according to the Sigma Diagnostics procedure 670-A. Mice were starved overnight and samples collected at approximately 10 A.M. the next day. Both measurements were normalized to plasma and urine creatinine levels, as determined by Sigma Diagnostics procedure 555-A.

Effect of B vitamins on wasting. Mice undergoing wasting were given an intramuscular injection in the abductor muscle of the thigh of vitamin at the following concentrations: cyanocobalamin at 25 μg/ml; niacinamide at 2 mg/ml; riboflavin at 2 mg/ml; thiamine at 12.5 mg/ml in distilled water or folate at 0.5 mg/ml in 1 M $NaHCO_3$. Dose was determined by body weight: animals weighing less than 20 g received 0.05 ml, with 0.05 ml more for every additional 5 g body weight. Each experiment employed three to four mutant and wild-type mice.

Vesicle preparation. Renal cortical microvillus membrane vesicles (BBMV) were prepared by the method of Guillery and colleagues (1994). For each preparation, 5–10 animals were sacrificed by cervical dislocation; kidneys were pooled and stored in ice-cold KHM buffer (80 mM HEPES, 40 mM KOH, 150 mM D-mannitol, pH 7.5). All subsequent manipulations were carried out at 4°C or on ice. A portion of the final membrane vesicle suspension was stored at −70°C and used later to determine membrane purity. The remainder was divided into two portions that were preequilibrated in the appropriate solutions overnight. Enrichment for brush border membrane was judged by gamma-glutamyl transpeptidase (γ-GTP) activity (George and Kenny 1973; Sigma Diagnostics Procedure 545-A), and ranged from 9- to 14-fold. The mutant γ-GTP specific activity averaged one-half that of wild type. Basolateral membrane contamination was examined by assaying Na^+/K^+-ATPase activity (George and Kenny 1973). The ratio of final to initial ouabain-sensitive ATPase activity varied from approximately 1.5 to 5 (Forbush 1983), with mutant membrane usually higher (data not shown). Protein content, determined by the method of Lowry and coworkers (1951) as modified by Peterson (1977), ranged from 8 to 20 mg/ml.

Uptake assays. After overnight preequilibration, uptake of radiolabeled isotopes into the membrane vesicles was assayed by a rapid filtration technique (Aronson and Sactor, 1975) at 20°C for the glutamine experiments and at 37°C for the Na^+/H^+ antiporter experiments. The compositions of the preequilibration and incubation solutions are described in the figure legends.

Transport was terminated by the addition of 2 ml of ice-cold stop solution at precisely timed intervals. The entire mixture was then poured over a prewetted 0.65-μM Millipore filter (DAWP). The filter was washed with an additional 4 ml of stop solution and placed in 5 ml of scintillation fluid. The isotope retained on the filter was determined by scintillation spectrometry. Nonspecific binding of isotope to the filter was determined by addition of stop solution directly to the radioisotope solution, followed by washing and filtration steps described above. This background was subtracted from the total activity for each uptake experiment.

Glutamine uptake experiments in which sodium was included in the incubation solution were designed to measure active uptake via sodium/amino acid symport (Evers et al. 1976; Reizer et al. 1994). Uptake in the absence of a sodium gradientoccurs by sodium-independent mechanisms, which are generally passive (Oxender and Christensen 1963; Mircheff et al. 1982; Christensen 1990). Sodium-dependent and passive uptake were measured in each vesicle preparation with the same preequilibration solutions and assay procedure.

Vesicles used to determine D-[^{3}H]glucose space were preequilibrated in 60 mM HEPES, 30 mM KOH, 100 mM potassium gluconate, 100 mM D-mannitol (pH 7.55). Eighty microliters of vesicle suspension were mixed with 80 μl of the same solution (without vesicles) containing 3.36 μM D-[^{3}H]glucose (final glucose concentration 1.68 μM) and incubated overnight at 4°C. Twenty-microliter samples were subjected to the same rapid filtration and washing as described above. Preequilibration solution was used as a stop solution. All assays were performed at least in triplicate for each experiment.

Data analysis and statistics. Data are presented as the average (±1 standard deviation), except for the glutamine uptake and Na^+/H^+ antiporter experiments. In each experiment, glutamine uptake and Na^+/H^+ antiporter-dependent Na^+ uptake are expressed as the uptake at each time point normalized to the average of the multiple uptake determinations for that genotype at 1 h of incubation. The averages of these normalized uptake values (±1 standard deviation) are presented. Data were compared by use of the unpaired Student's t-test.

Results

Biochemical screening. Preliminary experiments suggested that the delay in phenylalanine clearance in HPH2 mice was not due to a defect in phenylalanine catabolism (McDonald and Bode 1988; McDonald et al. 1990; Shedlovsky et al. 1993; data not shown). For assessment of whether the mutant was defective in amino acid transport, plasma and urine amino acids from mutant and non-mutant mice were quantitated. The concentrations of plasma amino acids were similar between the two groups, but the levels of most neutral amino acids in urine were greatly elevated in the mutants (Table 1). In particular, histidine and glutamine/asparagine were about 20-fold higher in the mutants than in the non-mutants; threonine, 10-fold higher; alanine, arginine, and serine, 6- to 7-fold higher. The levels of glycine, methionine, leucine, tyrosine, and phenylalanine were elevated 3- to 5-fold, and those of valine, cystine, tryptophan, lysine, and hydroxyproline 2- to 3-fold. Proline and citrulline were present in mutant urine but not detected in normal urine. The concentrations of all other amino acids were unaffected or elevated less than 2-fold.

Plasma and urine concentrations of glucose and phosphorus were determined for mutant and wild-type mice (three animals each). Glucose concentrations in both urine and plasma were normal, but fractional excretion of phosphorus was slightly lower in the mutant than in wild type (0.34 vs. 0.47) ($p < 0.05$; Student's *t*-test).

Amino acid transport in mutant kidney. The results of amino acid, glucose, and phosphorus screening all suggested a specific deficiency in amino acid transport in the mutant kidney. A direct measure of this activity was obtained by following the uptake of radiolabeled glutamine in BBMV of mutant and wild-type mice. Glutamine uptake (Fig. 1a) is presented as the amount of radioactivity at each time point normalized to that at 1 h, when the sodium

Table 1. Urine and plasma amino acid levels. Amino acid level is given as mmol amino acid/g creatinine; each value is the average (standard deviation) of four mutant and five non-mutant animals for urine, and four mutant and three non-mutant animals for plasma.

	Urine			Plasma		
Amino acid	mutant	non-mutant	mut./ non-mut.	mutant	non-mutant	mut./ non-mut.
His	1.91 (1.15)	0.09 (0.02)	21.2	44.6 (23.7)	48.9 (26.8)	0.91
gln/asn	9.22 (4.68)	0.51 (0.12)	18.1	396.2 (154.7)	367.7 (160.9)	1.08
thr	2.45 (1.46)	0.25 (0.10)	9.8	72.9 (50.0)	76.6 (34.4)	0.95
ala	2.18 (0.92)	0.31 (0.05)	7.0	196.2 (107.7)	210.3 (105.4)	0.93
arg	0.34 (0.15)	0.05 (0.05)	6.8	50.8 (41.0)	63.1 (38.7)	0.81
ser	1.20 (0.47)	0.20 (0.10)	6.0	78.5 (50.1)	69.1 (38.3)	1.14
cit	2.12 (1.48)	0.00	—	56.7 (15.4)	67.2 (33.3)	0.84
pro	0.11 (0.06)	0.00	—	54.7 (24.6)	57.5 (25.2)	0.95
met	0.46 (0.25)	0.11 (0.03)	4.2	37.6 (18.6)	39.3 (18.3)	0.96
phe	0.18 (0.06)	0.05 (0.01)	3.6	46.9 (20.0)	49.0 (24.6)	0.96
tyr	0.64 (0.29)	0.19 (0.02)	3.4	35.4 (16.9)	49.1 (25.0)	0.72
3-me-his	0.20 (0.19)	0.06 (0.03)	3.3	0.0	0.6 (0.24)	—
gly	1.80 (1.52)	0.57 (0.09)	3.2	110.7 (89.4)	133.7 (80.1)	0.83
leu	1.09 (0.32)	0.34 (0.09)	3.2	87.5 (56.5)	88.6 (44.8)	0.99
lys	0.58 (0.30)	0.20 (0.07)	2.9	205.5 (118.4)	223.6 (73.4)	0.92
Cys	0.63 (0.36)	0.26 (0.10)	2.4	4.9 (1.0)	6.4 (2.8)	0.77
trp	0.11 (0.07)	0.05 (0.02)	2.1	46.8 (23.0)	43.2 (16.1)	1.08
OH-pro	0.17 (0.13)	0.08 (0.05)	2.1	20.6 (6.3)	19.4 (21.4)	1.06
1-me-his	0.10 (0.03)	0.01 (0.02)	2.0	0.0	0.0	—
val	0.18 (0.05)	0.09 (0.04)	2.0	107.5 (73.2)	113.1 (50.2)	0.95
bala	0.29 (0.18)	0.16 (0.06)	1.8	0.0	0.0	—
ile	0.26 (0.18)	0.15 (0.02)	1.7	57.8 (37.9)	64.8 (32.7)	0.89
orn	0.48 (0.14)	0.33 (0.08)	1.5	51.1 (22.0)	13.0 (25.7)	3.93
asp	0.15 (0.04)	0.10 (0.05)	1.5	7.0 (3.8)	7.9 (5.0)	0.88
glu	0.03 (0.06)	0.09 (0.02)	1.4	27.5 (9.9)	29.6 (16.0)	0.93
tau	4.12 (2.2)	6.03 (0.87)	0.7	353.8 (51.9)	367.6 (168.6)	0.96

and amino glutamine gradients have equilibrated. When sodium was more concentrated outside the vesicle than inside, both mutant and wild-type vesicles initially accumulated more glutamine than at equilibrium (1 h) (Fig. 1a, upper two curves). This overshoot is indicative of transporter-mediated uptake (Heinz and Weinstein 1984). Wild-type vesicles concentrated glutamine more than did mutants for the first minute of incubation. By contrast, without a sodium gradient (Fig. 1a, lower two curves), no overshoot occurred and there was no difference in uptake between the two genotypes. Similar but less dramatic results were found when alanine was used as substrate (data not shown). Sodium-dependent alanine uptake was greater in wild-type than mutant vesicles through 30 s of incubation ($p = 0.05$ at 5 s). By contrast, in the absence of a sodium gradient there was neither an overshoot nor a difference between genotypes.

The specificity of these effects was demonstrated in assays for a second brush border transport activity, the Na^+/H^+ antiporter. Here, $^{22}Na^+$ uptake is driven by an outwardly directed proton gradient through Na^+/H^+ exchange (Kinsella and Aronson 1980; Guillery et al. 1994). Although Na^+/H^+ antiporter assays displayed the expected overshoot, peaking at 15 s (Fig. 1b), there was no difference between the two genotypes in Na^+/H^+ antiporter-dependent Na^+ uptake.

Vesicle size was measured as intravesicular D-[^{3}H]glucose space. Mutant vesicles averaged 2.15 (±0.12) μl/mg protein, and wild type averaged 1.90 (±0.14) μl/mg protein, indicating no significant difference, between the two genotypes ($p = 0.17$).

HPH2 as a model of Hartnup Disorder. In addition to the urine amino acid profile, the most distinctive phenotype associated with Hartnup Disorder is a niacin deficiency displayed by some patients. We had observed that HPH2 mice fail to thrive under certain dietary conditions. When maintained on the Purina 5001 (permissive) diet, homozygous mutants appear normal, despite the amino acid transport defect and associated hyperaminoaciduria. By contrast, on the Teklad Breeder Blox (restrictive) diet, the mutant displays a ''wasting syndrome'' characterized by weight loss and diarrhea. Within several weeks these animals die unless returned to the permissive feed before becoming moribund. This phenotype is fully penetrant in the BTBR congenic strain but varies in time of onset from 2 to 40 weeks, within an average of 12 (data not shown).

A protocol was developed to determine whether the wasting syndrome was reversed by niacin. Homozygous and heterozygous mutant mice were maintained on the permissive feed until they were several months old and then switched to the restrictive feed. All mice were weighed twice weekly, beginning 4–6 weeks before the change in diet. Wasting was defined as loss of more than 10% of the maximum weight attained after switching to the restrictive feed. When wasting had begun, mice were given an intramuscular injection of niacin, riboflavin, folate, thiamine, or cyanocobalamin (see Materials and methods). Non-mutant controls were treated at similar times. Reversal of wasting was defined as weight gain within 7 days after injection. It was found that only niacin could reverse the wasting syndrome (Fig. 2), and it had no significant effect on the weight of non-mutant mice.

The niacin deficiency symptoms associated with Hartnup Disorder are thought to be influenced by genetic background. Therefore, the wasting syndrome was examined in a mutant stock carried on a different genetic background (Symula et al. 1996). (SWR × BTBR)F_1 females carrying the mutation were backcrossed to BTBR mutant males. Homozygotes were identified phenotypically by phenylalanine clearance and genotypically by homozygosity for the BTBR allele of *D7Nds4*, a marker closely linked to *hph2* (Symula et al. 1996). It was found that mutants from this backcross population, carrying alleles from the SWR strain, developed wasting at a slower rate than those on the inbred BTBR background (Fig. 3): an average of 205 days (119≈305 days) in the backcross and 82 days (13–282 days) in the inbred backgrounds. Thus, the onset of wasting in the former class was significantly slower than that of the latter ($p = 1.2 \times 10^{-5}$, Wilcoxon Rank Sum Test). Notably, one of the 16 backcross mutants failed to develop wasting

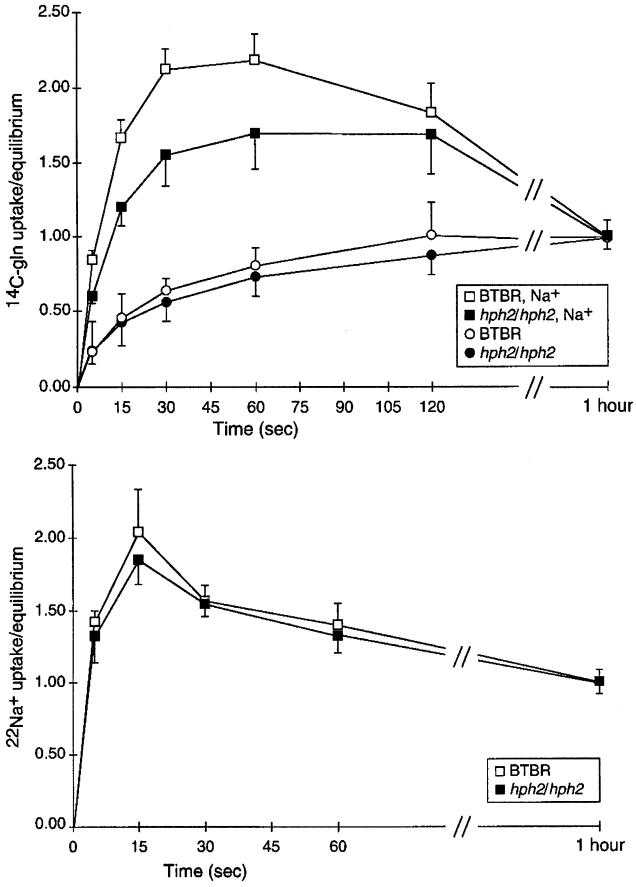

Fig. 1. Glutamine and Na^+ uptake by BBMV from mutant and wild type. Vesicle suspensions from cortical tissue were pooled from 5 to 10 animals of each genotype. Error bars represent one standard deviation. Note that the X-axis is not continuous, and uptake at time zero has been extrapolated to zero on the Y-axis. **a)** Vesicles were preequilibrated in a solution of 20 mM HEPES, 100 mM KCl, and 100 mM D-mannitol (pH 7.4). To measure sodium-dependent uptake of ^{14}C-glutamine (squares), 10 μl of vesicle suspension was mixed with 40 μl of incubation solution composed of 20 mM HEPES, 100 mM D-mannitol, and 100 mM NaCl, as well as 62.5 μM glutamine (pH 7.4, final glutamine concentration = 50 μM, final Na^+ = 80 mM). The same buffer was used to assay passive diffusion (circles), except that NaCl was replaced with 100 mM KCl. Stop solution contained 20 mM HEPES, 100 mM D-mannitol, and 100 mM KCl (pH 7.4). Each data point is the average of four experiments, expressed as the ratio of radioactivity at the indicated time to that at equilibrium (1 h). **b)** Vesicles were preequilibrated in a solution composed of 80 mM MES, 40 mM KOH, 50 mM potassium gluconate, and 100 mM D-mannitol (pH 6.10). Uptake of $^{22}Na^+$ as in part **a)** with an incubation solution containing 56 mM HEPES, 16 mM K^+, 50 mM gluconate, 103.2 mM D-mannitol, and 50 μM NaCl (pH 7.76). Stop solution was 10 mM HEPES and 154 mM LiCl (pH 7.50). Each data point is the average of three experiments expressed as the ratio of time indicated to that at 1 h.

for over one year, at which time the experiment was terminated. The mutant HPH phenotype of this mouse was confirmed by a phenylalanine clearance assay at the end of the experiment.

Discussion

The hyperaminoaciduria profile of HPH2 mice is similar to that characteristic of Hartnup Disorder (Baron et al. 1956; Seakins 1977; Scriver et al. 1987; Levy 1995): histidine, glutamine/asparagine, threonine, serine, arginine, and alanine are elevated 5- to 20-fold, while most of the other neutral alpha-amino acids as well as hydroxyproline, glycine, and lysine are elevated to a lesser extent. A high concentration of citrulline and a low level of proline

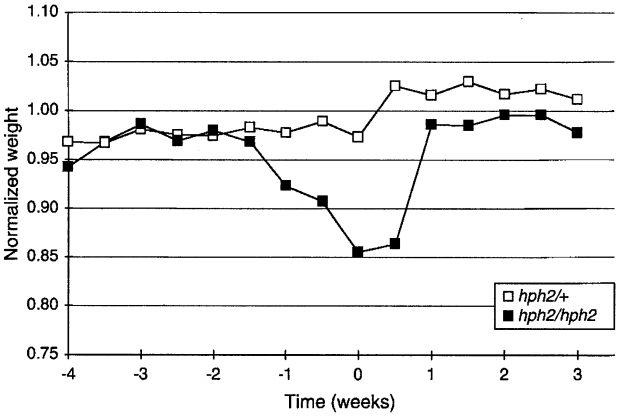

Fig. 2. Effect of niacin on wasting. Weights were normalized to the maximum weight of each mouse with the formula: 1-[(max − $weight_t$)/max]. $Weight_t$ is the weight recorded at time t. Maximum weight for each animal was that recorded after the diet change but before the onset of wasting. Time zero marks the onset of wasting as well as initiation of treatments. Since individual animals varied in the time at which wasting began, the curves have been aligned to permit comparisons. Each data point is the average of normalized weight. All animals were 3-4 months old at the start of the experiment. Intramuscular injections of niacin were given at weighted doses (see Materials and methods). n = 4 for the mutants and n = 3 for wild type.

are present in the urine of the mutant but not the wild type. Finally, the acidic amino acids, glutamate and aspartate, as well as ornithine, isoleucine, and the beta-amino acids β-alanine and taurine, are relatively unaffected.

Despite these similarities, the amino acid profile of HPH2 urine differs from that of the typical Hartnup patient. In general, for a given amino acid, excretion by HPH2 mice is less extreme than that seen in Hartnup Disorder. In particular, tryptophan and the branched chain amino acids are only moderately affected in the mouse mutant, but are greatly affected in Hartnup Disorder (Baron et al. 1956; Levy 1995). Conversely, arginine is among the amino acids most elevated in HPH2 urine, but is less affected than the neutral amino acids in Hartnup Disorder patients (Shih et al. 1971; Seakins 1977). Indeed, elevated cationic amino acids are not considered part of the Hartnup urine amino acid profile for this reason (Evers 1956; Shih et al. 1971; Seakins 1977).

These discrepancies between the HPH2 and Hartnup urine amino acid profiles could arise from differences in experimental

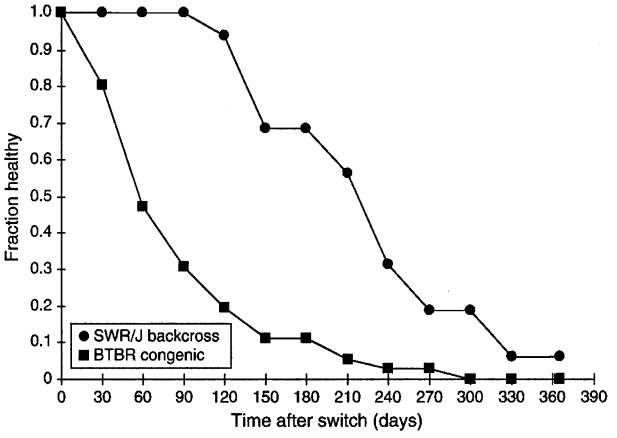

Fig. 3. Effect of genetic background on the wasting syndrome. Data are presented as the fraction of mutants not undergoing wasting, at each time point, for each background tested. Squares indicate inbred BTBR; circles indicate outbred mutants. n = 16 SWR/J backcross; n = 36 BTBR congenic.

method or diet. The Hartnup aminoaciduria is determined in urine collected over a 24-hour period or by calculation of renal clearance rates (Tarlow et al. 1972; Shih et al. 1971; Levy 1995). By contrast, the mouse profile presented in Table 1 was obtained from a single sample for each animal, collected at a standard time of day. Excretion over the course of the day may vary, and it is possible that in the mouse mutant a 24-hour average excretion would more closely resemble that of Hartnup Disorder.

If the HPH2 mouse strain is indeed a homolog of human Hartnup families, non-conserved genetic factors might explain the differences in phenotype, beyond the dietary and technical factors discussed above. For example, the wild-type gene products of the two species, though homologous, could differ in detailed specificity factors. Further, the *hph2* mutant allele studied here might not match any of the Hartnup family alleles. Finally, the set of compensatory systems directed by separate genetic loci may differ between the mouse and the human.

By contrast, we must also consider the possibility that the HPH2 mouse strain is not a genetic homolog of Hartnup Disorder but is affected in a gene with a similar role in amino acid transport. As discussed below, this question must be addressed first by genomic homology and ultimately by molecular identification of the *hph2* and Hartnup genes.

High concentrations of amino acids in urine, but not plasma, indicate a defect in kidney tubule transport (Silbernagl, 1992), which may be specific for amino acids or more general as in Fanconi syndrome. Fanconi patients are affected in transport of compounds other than amino acids (Bergeron and Gougoux 1989). In particular, glucose and phosphorus transport are strongly affected and appear at high levels in the urine. However, comparison of mutant and wild type revealed little difference in these compounds.

The BBMV experiments provide direct evidence of the amino acid transport defect suggested by this hyperaminoaciduria. Although the sodium-dependent uptake of glutamine and alanine was reduced in the mutant vesicles, considerable transport of these substrates is retained. Passive diffusion, Na^+/H^+ antiporter-dependent uptake of Na^+, and vesicle size are unaffected. Note that the latter experiment also serves as a control for non-specific binding in the active uptake experiment. These experiments, designed as a qualitative test for a transport deficiency in the mutant, do not discriminate between different amino acid transport systems (Christensen 1989, 1990) and do not describe the kinetic characteristics of the mutant defect.

From the urine profile, one might have expected a greater deficiency in uptake by the mutant vesicles. Glutamine and asparagine were not separated in the urine analysis. The combined excretion by the mutant is 18 times greater than that of the non-mutant. By contrast, glutamine uptake in vitro is reduced less than twofold. This discrepancy could result from differences between conditions in vivo and in vitro. For example, in the kidney tubule, urine amino acid concentrations will be influenced by competition among the various amino acids for these transporter systems (McKean et al. 1968; Olendorf 1973; Dillehay et al. 1980; Curriden and Englesberg 1981). Such effects may be enhanced by a reduction in the total transport state of the mutant. By contrast, the BBMV experiments measure transport of individual amino acids in the absence of cellular metabolic machinery. Further, the metabolic activity of the tubule cells themselves may influence the final urine amino acid profile.

The experimental conditions used to compare amino acid uptake in the mutant and wild type may not have been optimized, since ideal assay conditions could not be determined without knowing the characteristics of the presumed transporter. For example, one might have observed a greater difference between the two genotypes at substrate concentrations nearer the K_m of the affected systems. In addition, although the uptake experiments were performed at room temperature, it is possible that a temperature closer to that found in vivo would have increased the difference between the two genotypes.

Kidney brush border amino acid transport has not been examined in Hartnup Disorder. However, some patients display a defect in transport at the analogous intestinal brush border (Shih et al. 1971). Interestingly, the intestinal defect also affects transport of lysine and glycine (Shih et al. 1971), two amino acids not normally associated with the Hartnup urine profile. A similar defect in the kidney may be masked by compensating transporters.

Wasting. The weight loss and diarrhea, reported here in the mouse mutant, and the pellagra-like symptoms associated with Hartnup Disorder (Levy, 1995) appear to have a common basis and thus strengthen the resemblance of the mutant to Hartnup Disorder. When switched from a permissive to a restrictive diet, homozygous mutant mice begin wasting syndrome that can be reversed by niacin. [By contrast, a wasting syndrome in mice homozygous for a targeted disruption of the hepatic nuclear factor 1 gene (*Hnf1*) can be explained by glucose loss (Pontoglio et al. 1996)]. Further, we have presented evidence for an effect of genetic background on the wasting syndrome. On a hybrid background, mutants were less likely to develop diarrhea in conjunction with weight loss (data not shown), and the time of onset was considerably delayed relative to the inbred BTBR mutants.

Taken together, these results support the view that Hartnup Disorder depends on both environmental factors and genetic background. However, it is not clear how inadequate intake of niacin results in wasting. Although the niacin content is somewhat lower in the restrictive than in the permissive diets (67 and 95 ppm, respectively) this difference may not be sufficient to explain the wasting syndrome. Experiments with chemically defined diets would facilitate a study of the role of niacin or other compounds in wasting.

In summary, the experiments presented here indicate that HPH2 is indeed a model for Hartnup Disorder: the urine amino acid profiles are similar; both are deficient in brush border amino acid transport [Hartnup Disorder in at least the intestine (Shih et al. 1971; Tarlow et al. 1972) and HPH2 in at least the kidney]; and both display a niacin-reversible syndrome influenced by diet and genetic background. Identification of the mutated genes in these disorders will ultimately be required to test this hypothesis.

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