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Effect of Sodium Benzoate and Sodium Phenylacetate on Brain Serotonin Turnover in the Ornithine Transcarbamylase-Deficient Sparse-Fur Mouse

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Herein we examine the effects of sodium benzoate and sodium phenylacetate on feeding and central serotonin turnover in a child with citrullinemia and in an animal model of congenital hyperammonemia, the ornithine transcarbamylase-deficient sparse-fur (*spf/y*) mouse. In the child, when the benzoate/phenylacetate dosage was increased from 200 to 375 mg/kg/day each, feeding decreased. There was an accumulation of benzoate and phenylacetate in blood and cerebrospinal fluid as well as an increased concentration of 5-hydroxyindoleacetic acid, a neurochemical marker for serotonin turnover, in cerebrospinal fluid. In the mouse, sodium benzoate had a biphasic effect on both plasma ammonium levels and brain serotonin turnover. Two percent oral benzoate was associated with an increase in ammonium level, while a 3% dose led to a decrease in ammonium. There was a similar effect on serotonin turnover noted in both the hyperammonemic *spf/y* and control *CD-1/y* mice. Sodium phenylacetate did not have a consistent effect on serotonin turnover. The mechanism by which benzoate increases brain serotonin turnover appears to involve competition with tryptophan for albumin binding sites. This results in increased free tryptophan in serum and brain. We speculate that some of the clinical symptoms of benzoate intoxication may be a consequence of altered serotonin turnover in the brain. We suggest that drug levels be monitored during therapy. (*Pediatr Res* 23: 368–374, 1988)

Abbreviations

Trp, tryptophan
HIAA, 5-hydroxyindoleacetic acid
HPLC, high performance liquid chromatography
NE, norepinephrine
5-HT, serotonin
ANOVA, analysis of variance
GABA, γ-aminobutyric acid
CoA, coenzyme A

Sodium benzoate and sodium phenylacetate have proven effective in stimulating alternative pathways for waste nitrogen excretion in children with inborn errors of urea synthesis (1). This has resulted in prolonged survival. However, both intravenous drugs used to treat acute hyperammonemia and inadvertent oral overdoses have been associated with clinical toxicity including anorexia, irritability, lethargy, and coma (1, 2). Symptoms of intoxication in part simulate those of hyperammonemia.

The mechanism of these behavioral alterations is unclear. Bachmann *et al.* (3) reported increased brain uptake of Trp, the precursor of serotonin, in rats injected with 1 mmol/kg of sodium benzoate. Increased brain uptake of Trp and increased brain serotonin turnover have also been reported in experimentally induced and naturally occurring hyperammonemia (4–7). These findings, together with data showing an association of anorexia with increased serotonin turnover in children with urea cycle disorders (8), suggest that benzoate intoxication may involve altered serotonin turnover.

In studying the effect of benzoate and phenylacetate on serotonin turnover, we initially present a case report of a child with citrullinemia who developed toxic symptoms during high dose therapy and was found to have increased levels of the metabolite of serotonin, HIAA, in cerebrospinal fluid. We then examine in more detail the interaction of hyperammonemia and benzoate/phenylacetate treatment on brain serotonin turnover in the chronically hyperammonemic ornithine transcarbamylase deficient sparse-fur (*spf/y*) mouse.

METHODS

Biochemical methods. Plasma ammonium was measured by a microfluorometric adaptation of a glutamate dehydrogenase method as previously described (4). Whole blood and plasma (sodium heparin) were obtained by cardiac puncture under ether anesthesia using a 25-gauge needle. The mortality rate was <10% with this procedure. The blood was immediately placed on ice, and ammonium levels assayed on 50 μl plasma within 30 min. An aliquot of plasma was precipitated with 5% sulfosalicylic acid and stored at −4° C for subsequent analyses of amino acids by automated ion exchange column chromatography using a Beckman 6300 high pressure amino acid analyzer.

As heparin has been shown to decrease tryptophan binding to albumin, serum was used for tryptophan measurement (9). Free and bound tryptophan were immediately separated by ultrafiltration (Amicon Centrifree ultrafilter) using fixed angle rotor centrifugation at room temperature for 20 min at 2000 × *g*. Total Trp concentrations in serum and free Trp in ultrafiltrates were measured immediately by an adaptation of an HPLC isocratic method with fluorometric detection as previously de-

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scribed (4, 10). Using this approach, there was no difference in free Trp levels between paired samples measured with or without pH control.

Cerebrospinal fluid measurements from the child with citrullinemia were obtained at 0700 h because of diurnal variation. The measures were taken at a 1-month interval. On both occasions the child was receiving gastrostomy tube feedings by continuous infusion that stopped at 0200–0300 h and contained his total daily dose of benzoate and phenylacetate. During this time, he was also offered three meals a day using behavior management techniques as previously described (8).

In the mice, cortex was dissected by the method of Glowinski and Iverson (11), and 30 mg, diluted 1:20, was homogenized in a 0.1 N sodium acetate buffer, pH 4.95 for measurement of NE, HIAA, 5-HT, benzoate, phenylacetate, and Trp. Biogenic amines in cerebrospinal fluid and brain were determined by an isocratic HPLC method with electrochemical detection (4). Benzoate, phenylacetate, and hippurate concentrations were measured by an isocratic HPLC method with UV detection at 245 nm (1). Cortex, 100 mg, diluted 1:2, was homogenized in the same buffer and precipitated with 5% sulfosalicylic acid before amino acid analysis by column chromatography. Twenty-four-h urine specimens for measurement of hippurate were collected using a polycarbonate metabolic cage (Econo-Cage, AH Thomas Co.).

Animals studied. Sparse-fur (*spf/y*) male mice, hemizygous for X-linked ornithine transcarbamylase deficiency, were bred from the original Oak Ridge stock (12). The *spf/y* animals were the produce of matings of homozygous affected (*spf/spf*) females with normal (+/*y*) males from a *CD-1* background. All male progeny of these matings are affected with ornithine transcarbamylase deficiency. Liver of these *spf/y* animals were removed at the time of sacrifice and stored at −80° C for later determination of ornithine transcarbamylase activity. Ornithine transcarbamylase activity, measured by the method of Ceriotti (13), ranged from 3.6–8.8 μmol citrulline formed/h/mg protein in *spf/y* compared to our previously reported activity of 88–112 in *CD-1* mice (14). All animals studied were adults; however, the *spf/y* males were older (aged 2–6 months) than the control *CD-1* mice (Charles River; 2 months old).

All mice were fed an essential amino acid diet (Rogers-Harper, US Biochemical) providing 16% protein. For the study of long-term high dose oral administration, sodium benzoate (N.F.; Ruger Chemical Co.) was added to this amino acid mixture and made into pellets containing 0.1, 2, or 3% sodium benzoate (w/w). This resulted in mean sodium benzoate intakes of 0, 1.3, 1.7, and 3.0 g/kg/day (0, 9, 12, 21 mmol/kg/day) in *spf/y* mice. Serum sodium remained between 148–150 mEq/liter in all groups. Both *spf/y* and *CD-1/y* animals were divided into groups of five animals in each of the four treatment conditions (0, 1, 2, 3% benzoate). Two groups of *CD-1/y* mice were used, one was pair-fed with the matched *spf/y* mouse so that both groups ingested the same amount of benzoate; the other group was permitted to feed *ad libitum* in order to measure the effect of benzoate on food intake and weight. The biochemical studies were all performed on the pair-fed animals. All animals had a baseline period of 7 days receiving the amino acid diet, followed by a 5-day period receiving the amino acid diet plus the assigned dose of benzoate. Weights and food intake were measured daily during the study period.

To study the effects of bolus treatment used to treat acute hyperammonemic crises, sodium benzoate or sodium phenylacetate (Kendall-McGaw Pharmaceutical Co.) was injected intraperitoneally at doses of 1 or 5 mmol/kg. Animals injected with equimolar amounts of sodium acetate (Sigma Chemical Co.) were used as controls. Before injection, the animals were maintained on the amino acid diet for 7 days. The test compound was mixed with sterile water to give a final concentration of 100 mM for the 1 mmol/kg injections or 500 mM for the 5 mmol/kg injections. The animals were killed without anesthesia by decapitation 1 h after injection.

Statistical analysis. For the study of oral benzoate administration, there were four equally graduated benzoate dose groups (0, 1, 2, 3%) within each type of experimental mouse, *spf/y* and *CD-1/y*. An ANOVA with orthogonal contrasts was performed for the parameters measured in blood, urine, and brain of the eight study groups ($n = 5$/group). This analysis tested, in addition to the differences between the two experimental animals, the linear and nonlinear benzoate dose trends with respect to polynomial order of fit within each animal group.

For the study of intraperitoneal injections, a similar ANOVA analytic approach was used. However, different orthogonal contrasts were applied. These contrasts considered *spf/y versus CD-1/y* animals, differences within each animal group, and differences within and between acetate, phenylacetate, and benzoate treatments. A Bonferoni multiple comparison analysis was used to determine within group differences between specific benzoate dosages (15). For amino acid studies, significance was noted only at the $p < 0.01$ level because of the multiple comparisons of related compounds.

RESULTS

Case report. The patient is an 8-yr-old boy who was diagnosed as having citrullinemia during a 4-day episode of hyperammonemic coma beginning at 5 days of age. Peak ammonium level was 1550 μM (normal <50 μM). Plasma citrulline levels are chronically elevated, >2000 μM (normal 10–34 μM). Treatment consists of a nitrogen restricted diet (0.8 g/kg/day) supplemented with sodium benzoate 200 mg/kg/day, sodium phenylacetate 200 mg/kg/day, and arginine free-base 700 mg/kg/day. He has not subsequently had episodes of hyperammonemic coma, although he occasionally has elevations of plasma ammonium levels, in the range of 100–250 μM, as a result of excessive protein intake or an intercurrent infection. These episodes are manifest as irritability, lethargy, and vomiting and have responded within 24 h to treatment with intravenous arginine, sodium benzoate, and sodium phenylacetate.

The child is severely mentally retarded (mental age 2 yr) and refuses to eat food. This led to the placement of a gastrostomy feeding tube at 4 yr of age. He was recently admitted to the Kennedy Institute for behavioral management of his food refusal. Plasma ammonium level was 70 μM. An initial cerebrospinal fluid was obtained to measure levels of HIAA because of our previous observation of elevated levels in anorectic children with urea cycle disorders (8). We noted his HIAA level to be normal, 0.17 μM or 33 ng/dl (normal 0.11–0.23 μM). Once food refusal was behaviorally modified he began to eat spontaneously. It appeared therefore that he had food refusal rather than anorexia.

As a result of intermittent hyperammonemia during this hospitalization, we increased both his benzoate and phenylacetate dosage from 200 to 375 mg/kg/day, while retaining unchanged his protein (0.8 g/kg/day), caloric (1000–1300 cal), and arginine (700 mg/kg/day) intake. Three days after this increase, he developed symptoms of anorexia, irritability, and lethargy that simulated hyperammonemia. However, his ammonium level was 18 μM. Plasma levels of benzoate and phenylacetate showed a 3- to 4-fold increase comparing doses of 375 to 200 mg/kg/day (Table 1). Free Trp in serum also increased at the high dose benzoate and phenylacetate. We obtained repeat cerebrospinal fluid levels of HIAA, benzoate, and phenylacetate and found them all to be markedly increased compared to the initial cerebrospinal fluid on the lower benzoate/phenylacetate dosage. The clinical symptoms abated within 2 days of decreasing the dosage of benzoate and phenylacetate.

This observation suggested that clinical benzoate/phenylacetate intoxication may be associated with increased serotonin turnover, manifest as increased levels of HIAA in cerebrospinal fluid. This was studied further in an animal model of hyperammonemia, the *spf/y* mouse.

Animal studies. Effects of Oral Benzoate. We found that the

ornithine transcarbamylase-deficient *spf/y* mice had plasma ammonium levels that were approximately twice those found in unaffected *CD-1/y* mice (Table 2). Sodium benzoate treatment (1–3% added to the feeds) resulted in an increase in ammonium levels at the 2% dosage and a decrease at the 3% dosage in both *spf/y* and *CD-1/y* mice. This biphasic (nonlinear) treatment effect reached statistical significance, $p < 0.05$, in the *spf/y* mice.

In the pair-fed *spf/y* and *CD-1/y* mice, oral benzoate treatment resulted in significant increases in plasma benzoate and hippurate levels, and in urinary hippurate excretion. In the *spf/y* mice, plasma benzoate and hippurate levels and urinary hippurate excretion were linearly related to benzoate dose, $p < 0.001$. In the *CD-1/y* mice, a linear correlation ($p < 0.001$) was only demonstrated for urinary hippurate excretion. Benzoate and hippurate appeared to accumulate in plasma more in *spf/y* than in *CD-1/y* mice ($p < 0.05$). Between 20–29% of ingested benzoate was excreted as hippurate in urine over 24 h.

Table 3 lists levels of NE, HIAA, 5-HT, and Trp in cortex of *spf/y* and *CD-1/y* mice under the various oral benzoate intakes. *spf/y* mice had significantly higher levels of HIAA ($p < 0.001$), 5-HT, and Trp ($p < 0.005$) than did *CD-1/y* mice. There was no difference between *spf/y* and *CD-1/y* mice in levels of NE. Benzoate treatment had a significant effect on HIAA and Trp ($p < 0.001$) in *spf/y* mice. The effect of benzoate on HIAA and Trp fit best a biphasic (nonlinear) relationship ($p < 0.001$). There was a significant increase in these levels in animals receiving 1% benzoate and a significant decrease in animals receiving 3% benzoate. There was no benzoate effect on levels of NE. In *CD-1/y* mice, there was a similar, but less significant nonlinear effect of benzoate intake on HIAA levels ($p < 0.05$).

In the nonpair fed animals, under the various treatment conditions, *spf/y* mice ate less food per body weight and lost more weight than did *CD-1/y* mice ($p < 0.001$)((Table 4). In the *spf/y* mice, 2% benzoate intake (which was associated with a significant increase in ammonium levels) resulted in a significant weight loss and decreased food intake ($p < 0.05$). There was neither a significant change in ammonium levels nor in feeding or weight in *CD-1/y* mice.

Effect of intraperitoneal sodium benzoate and sodium phenylacetate. In a second experiment *spf/y* and *CD-1/y* mice received intraperitoneal injections of sodium benzoate or sodium phenylacetate. Plasma benzoate and hippurate levels were markedly elevated 1 h after injection of 1 or 5 mmol/kg sodium benzoate (Table 5). After the 1 mmol/kg dose, the benzoate level in brain, although detectable, was low. However, there was a large accumulation of benzoate in cortex after the 5 mmol/kg dose. Sodium phenylacetate resulted in an even greater accumulation of phenylacetate in plasma and cortex. There were no significant differences in levels comparing *spf/y* and *CD-1/y* mice. Animals treated with sodium acetate, as a control, had undetectable levels of benzoate, hippurate, and phenylacetate in blood and cortex. There was no mortality associated with the injections.

Plasma ammonium levels obtained 1 h after injection of sodium acetate, sodium benzoate, and sodium phenylacetate were significantly higher in *spf/y* as compared to *CD-1/y* mice ($p < 0.001$) (Table 6). Compared to acetate, neither intraperito-

Table 1. *Effect of varying dosage of benzoate/phenylacetate on cerebrospinal fluid HIAA levels in 8-yr-old with citrullinemia*

	Dosage (mg/kg/day)	Plasma levels (μM)				Cerebrospinal fluid (μM)		
	Benzoate/ phenylacetate	Ammonium	Free Trp	Benzoate	Phenylacetate	Benzoate	Phenylacetate	HIAA
	200	70	3.7	0.6	0.3	0.1	ND	0.17
	375	18	5.8	1.7	1.1	5.8	0.8	0.51
Normal range	0	<50	3–17 (17)*	ND†	ND	ND	ND	0.11–0.23 (16)*

* Reference.
† Not detectable.

Table 2. *Effect of oral benzoate 0–3% on ammonium and benzoate metabolism in pair-fed spf/y and CD-1/y mice [mean (n = 5 each group)]*

Benzoate intake	(%)	Plasma ammonium (μM)	Benzoate intake (μmol/24 h)	Urinary hippurate (μmol/24 h)	Plasma benzoate (μM)	Plasma hippurate (μM)
spf/y	0	275	0	Trace	4	ND
	1	275	290	59	14	48
CD-1/y	0	106	0	Trace	ND	ND
	1	132	300	90	7	42
	2	156	517	110	22	80
	3	132	704	149	6	30
Overall SD*		73	147	57	22	64
ANOVA				*p*		
Individual contrasts						
spf/y vs CD		<0.001			<0.05	<0.05
spf/y linear			<0.001	<0.001	<0.001	<0.001
Nonlinear		<0.05	<0.001	<0.001	<0.005	<0.005
CD-1/y linear			<0.001	<0.001		
Nonlinear			<0.001	<0.001		

* SD for error from ANOVA, df = 32.

Table 3. *Effect of oral benzoate (0–3%) on cortical biogenic amines in pair-fed spf/y and CD-1/y mice [mean, pmol/mg tissue (n = 5 each group)]*

	Benzoate intake (%)	NE	HIAA	5-HT	Trp
spf/y	0	2.24	2.82	3.44	37.6
	1	2.05	4.11	4.67	77.7
	2	2.37	2.91	3.34	46.3
	3	1.64	1.83	2.57	32.1
CD-1/y	0	1.56	1.45	2.69	30.0
	1	1.74	2.21	2.36	34.6
	2	2.86	2.08	1.81	41.5
	3	1.49	2.21	2.51	40.1
Overall SD		0.96	0.53	1.20	10.2
ANOVA			*p*		
Individual contrasts					
spf/y vs CD			<0.001	<0.005	<0.005
spf/y linear			<0.001		<0.05
Nonlinear			<0.001		<0.001
CD-1/y linear					
Nonlinear			<0.05		

Table 4. *Effect of oral benzoate (0–3%) on wt and feeding over 4 days*

	Benzoate intake (%)	Wt (g)	Wt change (g)	Daily food intake (g/wt)
spf/y	0	35	−0.8	0.12
	1	33	−0.4	0.15
	2	40	−3.8	0.08
	3	37	−2.2	0.11
CD-1/y	0	29	0.4	0.14
	1	28	3.2	0.16
	2	28	0.8	0.16
	3	27	2.0	0.14
Overall SD		3	2.3	0.03
ANOVA			*p*	
Individual contrasts				
spf/y CD		<0.001	<0.001	<0.001
t tests				
spf/y benzoate 2% *vs* benzoate 0%		<0.05	<0.05	<0.05
CD-1/y benzoate 2% *vs* benzoate 0%				

neal benzoate nor phenylacetate at doses of 1 or 5 mmol/kg was associated with a difference in ammonium levels 1 h after injection compared to acetate in either *spf/y* or *CD-1/y* animals.

Pretreatment free Trp levels in serum were similar in *spf/y* and *CD-1/y* mice. Benzoate treatment resulted in a significant increase in free Trp levels compared to acetate in *CD-1/y* mice ($p < 0.005$) (Table 6). This was dose related. There was a similar trend ($p < 0.09$) in *spf/y* mice. Compared to sodium acetate, sodium phenylacetate had no effect on free Trp levels.

Table 6 also shows that the effects of intraperitoneal benzoate and phenylacetate on biogenic amine and tryptophan levels in *spf/y* cortex are similar to those observed in cerebrospinal fluid of our child with citrullinemia. All levels were significantly higher in *spf/y* as compared to *CD-1/y* mice. Benzoate administration was associated with a dose-related increase in HIAA ($p < 0.001$) and Trp ($p < 0.05$) levels in *CD-1/y* mice. A similar but less significant effect was found in *spf/y* mice. Phenylacetate was not associated with changes in biogenic amines in *CD-1/y* mice. HIAA levels were modestly increased ($p < 0.05$) at the 5mmol/kg sodium phenylacetate dose compared to sodium acetate in *spf/y* mice.

Table 7 lists plasma amino acids after intraperitoneal benzoate. Only those amino acids that are relevant to the study are shown. There were no significant changes in the amino acids that are not shown. This convention is also used in Table 8. As expected in ornithine transcarbamylase deficiency, plasma glutamine levels were higher and citrulline levels lower in the *spf/y* as compared to the *CD-1/y* mice ($p < 0.001$). Additionally ornithine and lysine levels were lower in the *spf/y* animals. In the *CD-1/y* mice, benzoate treatment effects included lowering levels of serine, glycine, citrulline, lysine, and arginine ($p < 0.01$–$p < 0.001$); ornithine was increased. In *spf/y* mice, differences were only noted at the 5 mmol/kg benzoate dosage on lowering glutamine, glycine, methionine, and alanine. As expected, injections of sodium acetate did not have any effect on amino acid levels. Plasma amino acids were not measured in the phenylacetate-treated group because, unlike man who acetylates phenylacetate with glutamine, mice metabolize phenylacetate as the glycine conjugate and the amino acid pattern might have been misleading (18).

Amino acids in cortex were measured under the 5 mmol/kg sodium benzoate and 5 mmol/kg sodium acetate conditions (Table 8). Glutamine levels were elevated in cortex of *spf/y* as compared to *CD-1/y* mice ($p < 0.001$). Benzoate infusion was not associated with a change in glycine or GABA. The lack of effect of benzoate on cortical glycine reflects the fact that glycine does not cross the blood-brain barrier (19), so that its formation occurs within the central nervous system and is not affected by peripheral conjugation of glycine with benzoate.

DISCUSSION

Sodium benzoate and sodium phenylacetate appear to be effective and generally well tolerated in the acute and chronic management of congenital hyperammonemia; reports of toxicity have been rare (1, 2). However, when toxicity does occur, the behavioral complex mimics hyperammonemia. This is illustrated by our child with citrullinemia who presented with anorexia and irritability. We found, in both this child and in the ornithine transcarbamylase-deficient sparse-fur mouse, that the anorexia and weight loss was associated with increased 5-HT turnover. This was manifest as increased levels of Trp, the precursor of 5-HT, and of HIAA, its principal metabolite in cerebrospinal fluid of our patient and in cortex of the *spf/y* mouse.

This finding is compatible with the known role of 5-HT in appetite suppression (20), and suggests that the anorexia associated with benzoate overdose in children may be a consequence of increased central 5-HT turnover. This phenomenon was found both in the hyperammonemic *spf/y* mice and in the normoammonemic *CD-1/y* mice. In our patient this effect occurred at a benzoate dose less than twice that recommended for long-term therapy, 375 *versus* 250 mg/kg/day (1). In the mice the effect was consistently seen at doses of benzoate more than 3-fold higher than the recommended dose.

The mechanism of the increased serotonin turnover seems to involve competition of benzoate with Trp for albumin binding sites, resulting in increased free Trp levels in blood. We found that free Trp levels in serum obtained 1 h after intraperitoneal injections of benzoate, 5 mmol/kg, were increased in both *spf/y* and *CD-1/y* mice. Similar results were reported by Iwata *et al.* (21) who studied the effects of various drugs, including sodium benzoate, on free serum Trp levels in rats. Unlike other amino acids, Trp exists in both bound and free forms in blood (22).

Table 5. *Effect of intraperitoneal benzoate or phenylacetate on metabolite levels obtained 1 h later in plasma and cortex [mean (range)]*

	Plasma hippurate (μM)	Plasma benzoate (μM)	Cortical benzoate (pmol/mg tissue)	Plasma phenylacetate (μM)	Cortical phenylacetate (pmol/mg tissue)
*spf/y**					
Acetate (1 mmol/kg)	ND†	ND	ND	ND	ND
Acetate (5 mmol/kg)	ND	ND	ND	ND	ND
Benzoate (1 mmol/kg)	35 (0–163)	85 (0–380)	29 (0–143)	ND	ND
Benzoate (5 mmol/kg)	341 (224–504)	6362 (4473–8245)	2239 (1849–2892)	ND	ND
Phenylacetate (1 mmol/kg)	ND	ND	ND	523 (278–1044)	341 (131–581)
Phenylacetate (5 mmol/kg)	ND	ND	ND	9367 (7796–11072)	3832 (3162–4080)
CD-1/y					
Acetate (1 mmol/kg)	ND	ND	ND	ND	ND
Acetate (5 mmol/kg)	ND	ND	ND	ND	ND
Benzoate (1 mmol/kg)	74 (7–298)	30 (0–112)	ND	ND	ND
Benzoate (5 mmol/kg)	253 (140–472)	5627 (5070–6303)	2581 (2164–2931)	ND	ND
Phenylacetate (1 mmol/kg)	ND	ND	ND	562 (412–1076)	252 (139–526)
Phenylacetate (5 mmol/kg)	ND	ND	ND	8514 (7095–10388)	3344 (3032–3880)

* No significant differences between *spf/y* and *CD-1/y*.
† Not detectable.

Table 6. *Effect 1 h after intraperitoneal benzoate or phenylacetate on plasma ammonium, Trp, and cortical biogenic amines [mean (n = 5 each group)]*

	Plasma levels (μM)				Levels in cortex (pmol/mg tissue)			
		Free-Trp						
	NH4	Pre	Post	Change in free-Trp	NE	HIAA	HT	Trp
spf/y								
Acetate (1 mmol/kg)	171	15.3	12.5	−2.9	1.59	2.62	2.75	38.8
Acetate (5 mmol/kg)	200	15.0	15.9	0.9	1.70	2.09	3.03	37.6
Benzoate (1 mmol/kg)	206	12.7	14.4	1.7	1.85	2.51	3.15	30.1
Benzoate (5 mmol/kg)	192	12.0	16.1	4.1	1.68	3.96	2.78	57.3
Phenylacetate (1 mmol/kg)	164	10.9	14.1	3.2	1.56	2.48	2.11	24.2
Phenylacetate (5 mmol/kg)	198	13.1	14.2	1.1	1.47	4.03	2.24	30.7
CD-1/y								
Acetate (1 mmol/kg)	123	11.2	14.0	2.8	1.57	1.95	1.84	27.0
Acetate (5 mmol/kg)	144	11.6	12.0	0.4	1.25	1.67	1.33	27.8
Benzoate (1 mmol/kg)	119	11.9	14.1	2.2	1.31	2.58	1.98	28.5
Benzoate (5 mmol/kg)	132	10.4	25.5	15.1	1.32	3.12	1.68	44.7
Phenylacetate (1 mmol/kg)	156	14.2	15.9	1.8	1.55	2.23	2.01	17.5
Phenylacetate (5 mmol/kg)	202	12.5	14.8	2.3	1.71	2.85	2.45	22.4
Overall SD	55	3.1	37.3	5.0	0.41	0.90	0.80	11.2
ANOVA				*p*				
Individual contrasts								
spf/y vs CD-1	<0.001			<0.05	<0.05	<0.01	<0.001	<0.001
spf/y benzoate 1-5						<0.001		<0.001
spf/y AC vs benzoate						<0.005		
CD benzoate 1-5			<0.001	<0.001				<0.005
CD AC vs benzoate			<0.001	<0.005		<0.001		<0.05
Multiple comparisons (Bonferroni)								
spf/y AC5 vs PA5						<0.05		
CD AC5 vs PA5								

Free Trp represents approximately 15–25% of total Trp (23). There is evidence that it is the free Trp that is transported across the blood-brain barrier as substrate for 5-HT synthesis (17). As the activity of the 5-HT synthesizing enzyme, Trp hydroxylase, is not normally saturated in brain, increased brain Trp concentration would result in increased 5-HT synthesis and turnover (24).

In addition to benzoate competing for Trp binding sites, there may be a direct effect of benzoate on increasing brain Trp uptake. Bachmann *et al.* (3) found increased uptake of [^{14}C]Trp after a benzoate bolus in both normoammonemic and hyperammonemic rats.

Unlike benzoate, injections of sodium phenylacetate did not result in an increase in free Trp levels in serum or serotonin turnover in cortex in either *spf/y* or *CD-1/y* mice. These results should be interpreted with some caution as phenylacetate is metabolized differently in rodents than in man (18).

In addition to studying the effect of benzoate on serotonin turnover in *spf/y* mice, we also approached the issue of whether benzoate can accentuate hyperammonemia. We noted that ben-

Table 7. *Effect of intraperitoneal benzoate on plasma amino acids obtained 1 h later [mean (SD) μM]*

	Ser	Glutamate	Glutamine	Gly	Ala	Cit	Orn	Lys	Arg
spf/y									
Acetate (1 mmol/kg)	135	59	740	338	308	22	66	288	46
Acetate (5 mmol/kg)	133	76	561	585	513	37	109	409	50
Benzoate (1 mmol/kg)	186	8	653	871	634	45	127	538	82
Benzoate (5 mmol/kg)	60	64	400	140	139	20	67	228	44
CD-1/y									
Acetate (1 mmol/kg)	199	48	527	905	606	126	119	843	129
Acetate (5 mmol/kg)	255	82	424	697	536	128	98	583	122
Benzoate (1 mmol/kg)	126	81	403	463	551	85	202	544	13
Benzoate (5 mmol/kg)	110	103	289	318	222	100	141	424	48
Total SD	71	37	113	273	205	27	45	177	35
ANOVA				*p*					
Individual comparison									
spf/y vs CD			<0.001			<0.001	<0.005	<0.001	
spf/y									
Acetate (1 *vs* 5)									
Benzoate (1 *vs* 5)			<0.01	<0.001	<0.005				
Acetate *vs* benzoate									
CD-1/y									
Acetate (1 *vs* 5)									
Benzoate (1 *vs* 5)									
Acetate *vs* benzoate	<0.005			<0.005		<0.01	<0.005	<0.01	

Table 8. *Effect of intraperitoneal benzoate on brain amino acids [mean (nmol/mg tissue)]*

	Ser	Glutamate	Glutamine	Gly	Ala	Cit	GABA	Orn	Lys	Arg
spf/y										
Acetate (5 mmol/kg)	368	4250	2835	333	371	ND	881	13	103	41
Benzoate (5 mmol/kg)	353	3720	2979	415	329	ND	1039	18	122	42
CD-1/y										
Acetate (5 mmol/kg)	389	3679	1841	353	399	ND	926	16	111	45
Benzoate (5 mmol/kg)	418	3900	1778	417	356	ND	1067	12	135	45
Overall SD	64	523	515	50	56		156	8	19	15
ANOVA					*p*					
Individual contrasts										
spf/y vs CD			<0.001							
spf/y AC vs benzoate										
CD AC vs benzoate										

benzoate can accentuate hyperammonemia. We noted that benzoate had a nonlinear (biphasic) effect on plasma ammonium levels. Mice receiving 2% benzoate orally (12 mmol/kg/day) had higher ammonium levels than mice receiving both higher (3%) and lower doses (0 or 1%). The lowest ammonium levels occurred at the 3% benzoate dose.

These findings support a paradoxical effect of benzoate on ammonium levels (25). At certain doses it may potentiate hyperammonemia, whereas at other doses it alleviates hyperammonemia. A possible explanation is that at a dose that leads to benzoate accumulation but little hippurate excretion, the effect of benzoate is to inhibit urea synthetic activity (26). There is evidence that the intermediate, benzoyl CoA, accumulates at high benzoate doses and that CoA-dependent processes are impaired as a result (27). One such process is the formation of N-acetyl glutamate, the activator of the first enzyme in the urea cycle, carbamyl phosphate synthetase. However, this inhibitory effect is counterbalanced at a dose that results in a significant alternate pathway of waste nitrogen excretion as hippurate. Accentuation of hyperammonemia should not occur at the benzoate doses recommended for treatment of urea cycle disorders.

This biphasic effect of benzoate administration would be accentuated in mice, where excretion of hippurate is half than in man (2, 28). A lack of benefit of intravenous benzoate on ammonium levels in mice may be related to the short time (1 h) between injection and death. Benzoate levels in plasma of mice during oral therapy were comparable to those reported during long-term benzoate therapy in children with urea cycle disorders (1).

The biphasic effect of benzoate on ammonium levels was reflected in brain 5-HT turnover. Levels of Trp and HIAA in the cortex increased with 1–2% benzoate doses and decreased at 3% benzoate intake. It is also of note that the hyperammonemic *spf/y* mice consistently had higher 5-HT turnover than did the *CD-1/y* mice. These combined data suggest that hyperammonemia and benzoate increase 5-HT turnover in a synergistic fashion.

As a result of these studies, we suggest that the range of benzoate that may be given without clinical toxicity may be rather narrow. Further, some of the clinical findings of benzoate intoxication, including anorexia and lethargy, may be a consequence of increased 5-HT metabolism. It would seem appropriate to periodically measure benzoate levels in plasma, especially

during high dose oral therapy or with intravenous bolus treatment of acute hyperammonemia in children with inborn errors of urea synthesis.

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