Fate of Anthocyanins and Antioxidant Capacity in Contents of the Gastrointestinal Tract of Weanling Pigs Following Black Raspberry Consumption

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Many fruits are rich in anthocyanins (ACNs). ACNs have high antioxidant capacity, but because of their apparent low bioavailability, their possible roles in health promotion in vivo are still in question. The objectives of these studies were to determine the fate of ACNs within the gastrointestinal (GI) tract and the effect on the bioavailability and subsequent metabolism of ACNs. Five weanling pigs were fed freeze-dried black raspberry (*Rubus occidentalis* L.) powder by oral administration, which provided 1146.1 ± 44.6 μmol TE of oxygen radical absorbance capacity with fluorescein as a fluorescent probe ($ORAC_{FL}$) per kg and 50.5 ± 3.7 mg per kg total ACNs. After 4 h, the pigs were sacrificed and the contents of five GI segments (duodenum, jejunum, ileum, cecum, and colon) were collected and analyzed for their total antioxidant capacity (TAC, measured as $ORAC_{FL}$) and ACNs. The recoveries of TAC and total ACNs were 46.5 ± 3.5 and $41.7 \pm 4.9\%$, respectively. Both total ACNs and TAC were recovered primarily in the ileum, cecum, and colon at 4 h after a meal. Cyanidin aglycone with different sugar moieties showed significant differences in their recovery within the GI tract with sambubiose > sambubiose-rhamnose = rutinose $\gg$ glucose. Recovery of ACNs within the GI tract was positively and linearly associated with urinary ACN recovery, which suggests that stability within the GI tract and not decreased absorption accounts for the increased recovery. The environment of different segments of the GI tract may determine the stability of individual ACNs. Complex ACNs containing di- or triglycosides disappeared more slowly in the GI tract than simple ACNs such as a monoglycoside. TAC and total ACNs remained high 4 h after feeding, which indicates that ACNs provide significant antioxidant protection in the environment of the gut epithelium.

KEYWORDS: Anthocyanin; antioxidant; black raspberry; gastrointestinal tract; metabolism

INTRODUCTION

High intakes of fruits and vegetables are believed to be related to a low incidence of heart disease and cancer, based upon a number of epidemiological studies and clinical trials. As one of the major groups of dietary phytochemicals, anthocyanins (ACNs) are distributed in many fruits, with berries being particularly rich in ACNs (*1−3*). ACNs are water-soluble glycosides and acylglycosides of anthocyanidins, which are polyhydroxyl and polymethoxyl derivatives of 2-phenylben-zopyrylium (flavylium) cation (*4*). ACNs have gained considerable attention recently for their possible health effects, and several studies have been completed recently investigating the bioavailability of ACNs (*5−19*). Much lower apparent absorption rates and differing metabolic pathways have been observed as compared to those of the other flavonoids, which may partly be due to ACN's unusual cation structures at low pH. Because of their apparent low bioavailability and instability at pH > 3,

their contribution to health benefits in vivo remains to be elucidated.

It is known that flavonoids undergo transformation by intestinal microorganisms, and various products of degradation may be formed during this process (*20, 21*). Most recently, several in vitro studies have demonstrated several breakdown products and metabolites of ACNs by human gut microflora (*22, 23*). These in vitro studies provide some information relative to understanding the poor bioavailability of ACNs, but they do not completely describe the situation in vivo.

Black raspberries (*Rubus occidentalis* L.) (BRBs) are rich in ACNs and have a high antioxidant capacity. There are seven ACNs that have been detected in BRB (*3*). Five of them predominate; they are as follows: cyanidin-3-rutinoside (Cy-3-rut) > cyanidin-3-sambubioside-5-rhamnoside (Cy-3-sam-5-rha) > cyanidin-3-glucoside (Cy-3-GLC) > cyanidin-3-sambubioside (Cy-3-sam) > pelargonidin-3-rutinoside (Pg-3-rut). BRBs are one of only a few berries that contain Cy mono-, di-, and triglycosides (*2*). Cy-based ACNs are the most abundant ACNs in nature (*1*). From our previous study, the sugar moiety

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plays an important role in the bioavailability of ACNs (*8*, *24*). Thus, BRB is an excellent experimental material with which to study the impact of different sugar moieties on the fate of ACNs in the gastrointestinal (GI) tract. Recent studies have also demonstrated that ACNs or other phytochemicals such as ellagic acid, flavonones, and procyanidins in BRBs may contribute to the protection observed in several different types of cancer models (*25*−*29*). BRBs inhibit the development of oral, esophageal, and colon cancer in rodents, and an extract of BRBs inhibited benzo[*a*]pyrene-induced cell transformation of hamster embryo fibroblasts. However, the actual active components have not been confirmed. Considering the extremely high concentrations in BRBs and evidence from published data (*30*−*33*), ACNs may be at least partly responsible for the observed anticancer activities.

The objectives of this study were to (i) investigate the fate of ACNs and their contribution to the total antioxidant capacity (TAC) within the GI tract following BRB feeding and (ii) determine the stability of ACNs within the GI tract and the effect on absorption/metabolism.
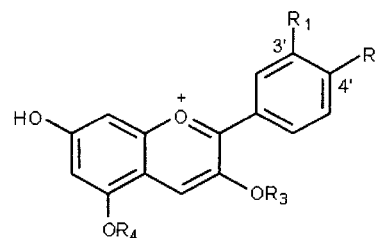
MATERIALS AND METHODS

Chemicals and Materials. The 3-*O*-*β*-GLC of Pg, Cy, peonidin, delphinidin, petunidin, and malvidin [six mixed ACN standard, high-performance liquid chromatography (HPLC) grade], were obtained from Polyphenols Laboratories (Sandnes, Norway). Methanol was obtained from Fisher Scientific (Fair Lawn, NJ), formic acid was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI), and trifluoroacetic acid (TFA) was obtained from Sigma Chemical Co. (St. Louis, MO). Sep-Pak Vac RC (500 mg) C_{18} Cartridges for solid phase extraction (SPE) were purchased from Waters (Milford, MA).

Experimental Materials. Freeze-dried BRB powder was provided by the Oregon Raspberry and Blackberry Commission as reported previously (*3*, *34*).

Animals and Study Design. All animal protocols were approved by the UAMS Animal Care and Use Committee. Healthy pigs (Hampshire/Duroc Cross) (*n* = 5, 21 days of age) were purchased from a local swine producer and brought to the Arkansas Children's Nutrition Center animal facility and allowed to adapt for a period of 7 days before surgery. At surgery, an indwelling cannulae was placed in the femoral artery. Four days before administration of berry powder, the pigs were fed a purified diet (*8*), which was free of any polyphenolic or flavonoid-like compounds. At the time of the experiment, the pigs weighed 16.3 ± 5.9 kg (mean ± SD; *n* = 5).
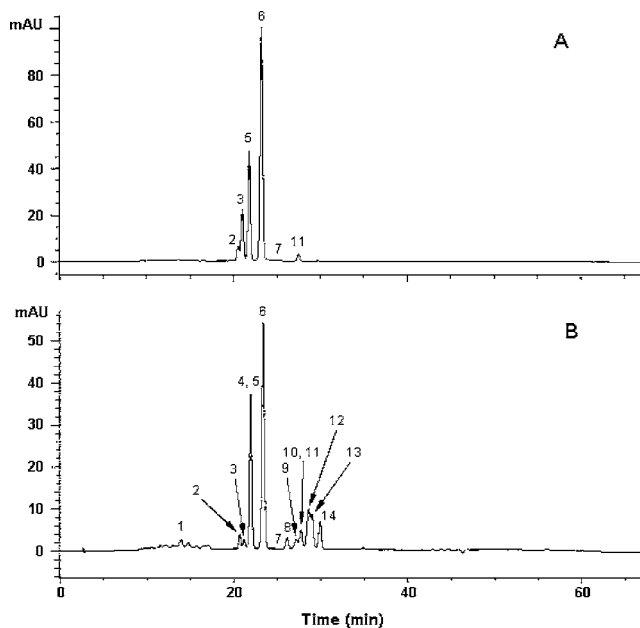
The pigs were placed in a metabolic cage and were fasted overnight with water freely available before the experiment. A baseline urine sample was collected in the morning. Freeze-dried BRB powder was mixed with water (1:3, w/w) and was given via gastric intubation. This dose provided 1146.1 ± 44.6 *μ*mol Trolox equivalents (TE) of oxygen radical absorbance capacity with fluorescein as fluorescent probe (ORAC_FL) per kg body weight (BW) and 50.5 ± 3.7 mg per kg BW of total ACNs (mean ± SD; *n* = 5). Urine samples were collected from pigs before and between 0 and 2 and 2 and 4 h after consumption of the berry powder. The urine samples were treated with 0.44 mol/L TFA as reported previously (*8*). After 4 h, the pigs were sacrificed by euthanasia with Nembutal given IV. Five sections of the GI tract, namely, duodenum, jejunum, ileum, cecum, and colon, were collected. The contents of each section were flushed out with saline. The contents with saline were weighed and mixed. A portion of each sample of gut contents was taken, and 0.44 M TFA was added to a concentration of 20% of the sample to stabilize the ACNs. Both urine and gut content samples were stored at −70 °C until analysis.

Sample Preparation. *Berry Sample.* BRB powder was extracted on an ASE 200 accelerated solvent extractor (Dionex Corp., Sunnyvale, CA) for lipophilic and hydrophilic ORAC_FL assay, as reported previously (*35*, *36*). For ACN analysis, the extraction and sample preparation were as published previously (*34*).
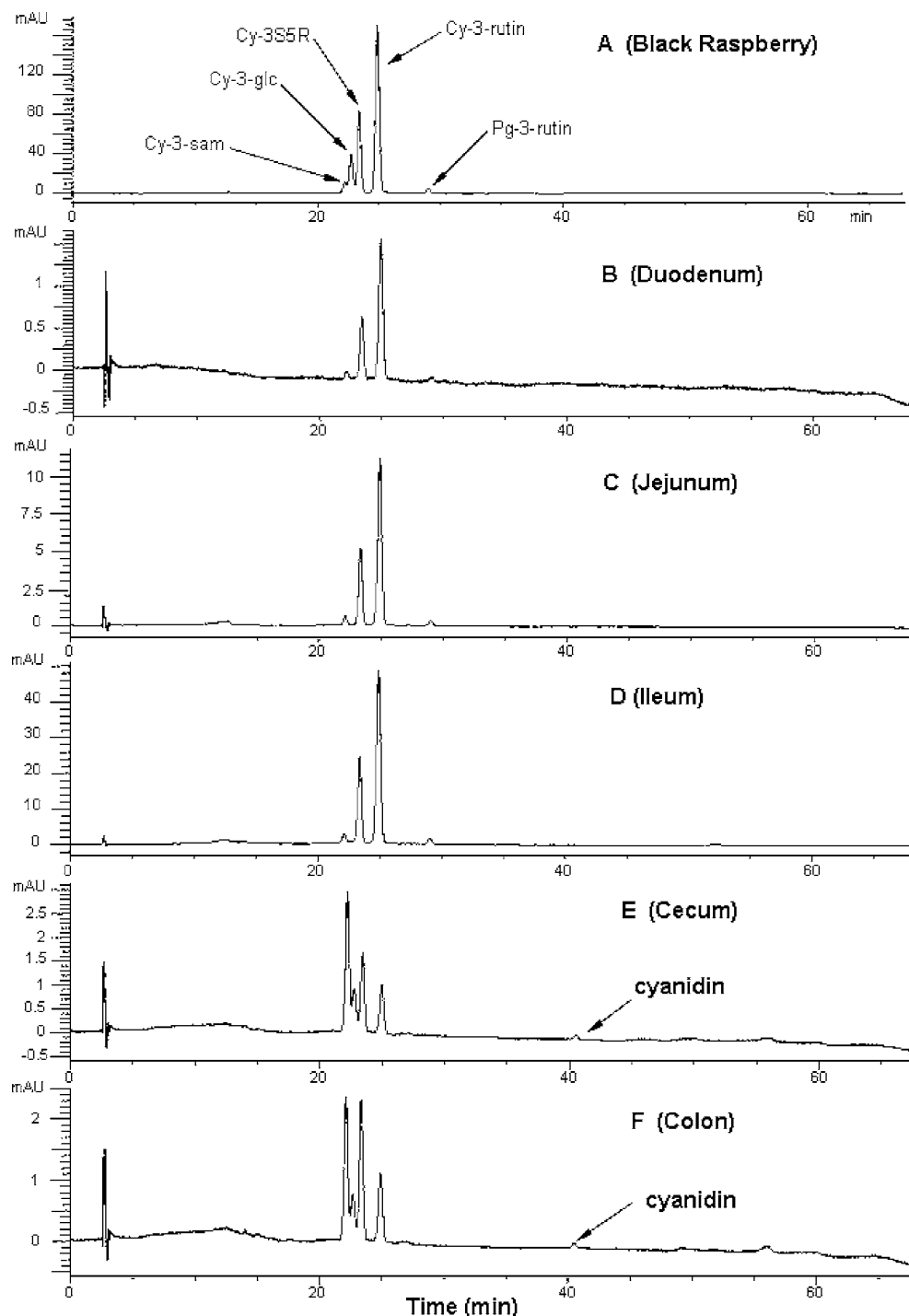
Figure 1. Chemical structures of major anthocyanins in the black raspberry and their metabolites in the urine organized by peak numbers corresponding to that in **Figure 2** (Abbreviations are in accordance with those in the text; sambubiose, 2-*O*-*β*-D-xylosyl-D-glucose; rutinose, l-*O*-α-L-rhamnosyl-α-D-glucose).

ANTHOCYANIN	R₁	R₂	R₃	R₄
2. Cyanidin-3-sambubioside	OH	OH	Sam	H
3. Cyanidin-3-glucoside	OH	OH	GLC	H
5. Cyanidin-3-sambubioside-5-rhamnoside	OH	OH	Sam	Rha
6. Cyanidin-3-rutinoside	OH	OH	Rut	H
7. Pelargonidin-3-glucoside	H	OH	GLC	H
9. Isopeonidin-3-glucoside	OH	OMe	GLC	H
10. Peonidin-3-glucoside	OMe	OH	GLC	H
11. Pelargonidin-3-rutinoside	H	OH	Rut	H
13. Isopeonidin-3-rutinoside	OH	OMe	Rut	H
14. Peonidin-3-rutionside	OMe	OH	Rut	H

Figure 2. RP-HPLC chromatograms of ACNs in BRB (**A**) and representative urine samples 2–4 h after feeding BRB (**B**) detected at 520 nm. Peak identities and their MS data are shown in **Table 1**.

Urine. Six milliliters of treated urine sample (5 mL of urine plus 1 mL of 0.44 M TFA) was passed through a Sep-Pak C_{18} SPE cartridge as described previously (*5*−*7*). After SPE treatment, the acidic methanol solutions of urine samples were evaporated to dryness with a SpeedVac (SC210A, ThermoSavant, Holbrook, NY) and redissolved in 500 or 200 *μ*L of a 5% formic acid/methanol solution. After filtration with a syringe filter (0.22 *μ*m, Phenomenex, Torrance, CA), the solution was injected into the HPLC-ESI/MS/MS system for analysis of ACNs. ACN standards were dissolved in acidic methanol to make calibration solutions for quantification and identification purposes.

Gut Contents. Two grams of contents from each GI tract segment was weighed and then extracted using acetone/water/acetic acid (70: 29.5:0.5, v/v, AWA) twice (10 mL × 2). The mixture of sample and solvent was centrifuged at 4550*g* for 10 min, and the supernatants from

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Figure 3. RP-HPLC chromatograms of ACNs in BRB (**A**) and representative gut content samples from five segments 4 h after feeding BRB (**B**–**F**) detected at 520 nm.

the two extractions were combined. The combined supernatant was transferred to a 25 mL volumetric flask, and AWA was added to make up the final volume to 25 mL. This stock solution was used for ORAC$_{FL}$ assay and ACN analysis.

Analysis of ACNs in BRB and Pig Urine. The analysis of ACNs in urine from the BRB-fed pigs was carried out on an Agilent series 1100 HPLC system including an autosampler, a binary pump, Zorbax SB-C$_{18}$ column (4.6 mm × 250 mm), and a diode array detector (Agilent Technologies, Palo Alto, CA). Low-resolution electrospray mass spectrometry was performed with an Esquire-LC Mass Spectrometer (MS) (Bruker Daltonics, Billerica, MA). Experimental conditions were kept the same as described previously (*7*, *8*).

ORAC$_{FL}$ Assay. Both hydrophilic and lipophilic ORAC$_{FL}$ assays of BRB were carried out on a FLUOstar Galaxy plate reader following

the procedure based on modified ORAC$_{FL}$ method (*37*, *38*). Extracts of gut contents were assayed for hydrophilic ORAC$_{FL}$ only, and this value was considered to be the TAC as BRBs have less than 1% of the TAC as lipophilic antioxidants.

Statistics. All data with a sample number equal or larger than three were expressed as means ± SEM if not mentioned specifically. Charts and graphs were made using Sigma Plot 2001 (SPSS Inc. Chicago, IL).

RESULTS

ACN Concentration and Antioxidant Capacity of BRB. BRB was found to contain seven ACNs with two different anthocyanidins, Cy and Pg (*3*). The total ACN content in BRB

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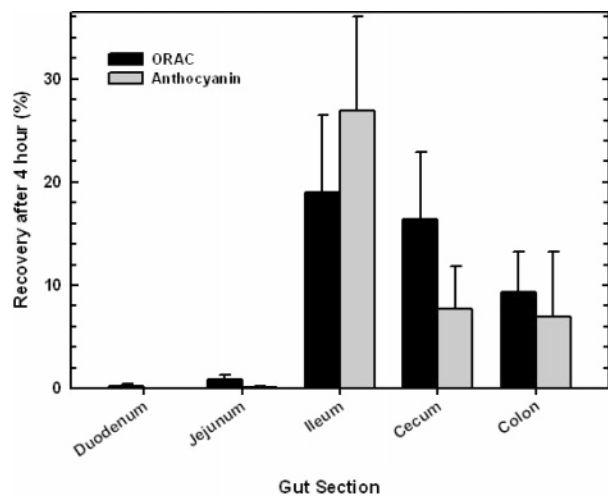
Figure 4. Recovery of total ACNs and TAC (ORAC$_{FL}$) of five segments of GI tract after 4 h of BRB feeding.

Figure 5. Recovery of individual ACNs in the whole GI tract 4 h after feeding of BRB.

powder was 43.6 mg/g dry weight (DW). The concentrations of the six major ACNs were as follows: 1.3 mg/g of Cy-3-sam (peak 2), 4.1 mg/g of Cy-3-GLC (peak 3), 12.1 mg/g of Cy-3-sam-5-rha (peak 5), 25.2 mg/g of Cy-3-rut (peak 6), 0.1 mg/g of Pg-3-GLC (peak 7), and 1.0 mg/g of Pg-3-rut (peak 11) (DW; **Figures 1 and 2A**), respectively. The hydrophilic and lipophilic ORAC$_{FL}$ values were 901.4 and 5.0 μmol TE/g DW, respectively. The sum of these two values (906.4 μmol TE/g, DW) was considered to represent the TAC of BRB using a peroxyl radical generator.

Identification and Quantification of ACNs in Gut Contents. The representative profile of ACNs in the contents of five different segments of GI tract (duodenum, jejunum, ileum, cecum, and colon) of pig following BRB feeding is shown in **Figure 3B−F**. Identification of ACNs in gut contents was accomplished by comparing their retention times and MS data with those of BRB. Only ACNs in the BRB as well as a trace amount of Cy were found (i.e., absorption spectra with maximum near 520 nm). Four hours after BRB feeding, the recovery of total ACNs from the whole GI tract was 41.7 ± 4.9% and recovery in the different GI segments is shown in **Figure 4**. By 4 h, most of the meal was present in the ileum or beyond.

Of the five predominant ACNs in BRB, Cy-3-sam had the highest recovery (% of dose), 78.2 ± 17.2%, whereas Cy-3-GLC exhibited an extremely low recovery of only 1.7 ± 1.0% (**Figure 5**). The recoveries of the three other ACNs were 40−50% (46.4 ± 3.5% for Cy-3-sam-5-rha, 43.7 ± 6.4% for Cy-3-rut, and 50.0 ± 8.0% for Pg-3-rut).

TAC of Gut Contents. TAC of gut contents from pigs following BRB feeding was measured as hydrophilic ORAC$_{FL}$ 4 h after feeding. The recovery of TAC in the whole GI tract across five pigs was 46.5 ± 3.5%; the recovery of TAC in each segment of the GI tract is shown in **Figure 4**.

ACNs and Their Metabolites in Urine. A representative ACN profile in pig urine following BRB feeding is shown in **Figure 2B**. Peak identification and assignment were based on the comparison of their retention time or MS data with that of BRB, standards, or published data. ACNs in the urine were quantified on the basis of their corresponding anthocyanidin-3-GLC (**Table 1**). For coeluting peaks, the percentage of the peak area that was attributed to each peak was determined by the peak area of the extracted ion chromatogram (EIC) as described in our previous paper (8).
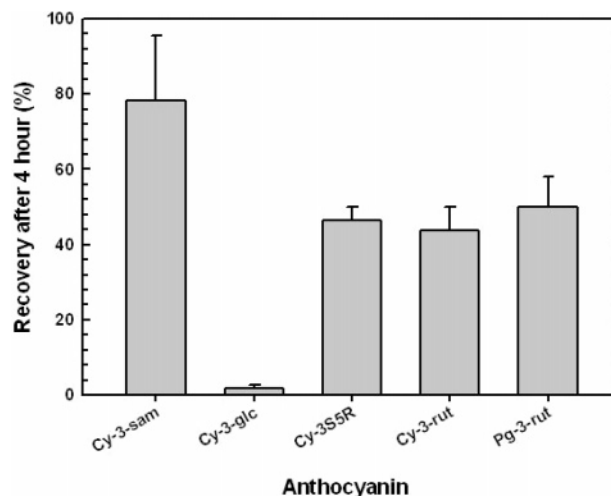
A total of 14 ACN-based compounds were detected in urine samples; six of them were identified as parent compounds, which existed in the BRB (peak 2, Cy-3-sam; peak 3, Cy-3-GLC; peak 5, Cy-3-sam-5-rha; peak 6, Cy-3-rut; peak 7, Pg-3-GLC; and peak 11, Pg-3-rut) (**Figure 2B**). Eight other ACNs were determined to be metabolites of one of the six parent ACNs. Peak 1 had a molecular ion mass-to-charge ratio (m/z) of 639 and two fragment ions m/z 477 and 301 and was identified as peonidin-3-GLC monoglucuronide by comparing its MS data and retention time with our previous paper (24). Similarly, other metabolites were identified as follows: peak 4, Cy monoglucuronide; peak 8, Pg monoglucuronide; peak 9, isopeonidin-3-GLC; peak 10, peonidin-3-GLC; peak 12, peonidin monoglucuronide; peak 13, isopeonidin-3-rut; and peak 14, peonidin-3-rut (**Figure 1**). Their concentration, recovery, and MS data are summarized in **Table 1**, and the relative proportions (% of dose of parent ACN) of the different metabolites are presented in **Figure 7**.

DISCUSSION

Berries with dark color have been found to contain high quantities of ACNs and show strong in vitro antioxidant capacities, being much higher than in most common foods (3, 36). BRB is one of several berries with high antioxidant capacity and ACN concentrations. As one of the major groups of phytochemicals in BRB, ACNs may be associated with its observed cancer chemoprevention (33, 39). Prevention of oxidative stress may be one of the possible mechanisms for the observed cancer prevention (33, 39). Several studies have been done to evaluate changes in antioxidant capacity in plasma or urine with fruit or berry consumption (Prior, R. L. et al. Unpublished data). However, no published research is available, which evaluates the changes in antioxidant capacity of the contents of the GI tract. Dietary antioxidants provide a first line of defense against oxidative events within the GI tract and thus may be important in prevention of certain diseases such as colon cancer. Therefore, investigation of the fate of ACNs and evaluation of antioxidant changes in the GI tract is important relative to the health effects of berries.

ACN Recovery in the Gut. The total recovery of ACNs and antioxidant capacity were similar (41.7 vs 46.5%). In addition, recoveries of total ACNs and TAC exhibited a very similar pattern in the different segments of the GI tract (**Figure 4**). After 4 h of BRB feeding, the majority of the ACNs and TAC moved

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Table 1. Identification of Original ACNs and Their Metabolites and Their Recovery in Urine after BRB Consumption[a]

peak no.	RT[b]	MS (*m/z*)	MS/MS (*m/z*)	compound	dose, parent ACN (μmol/kg BW)	ACN in urine (nmol/kg BW)	recovery in urine[c] (%)
1	13.9	639	477/301	peonidin-3-GLC monoglucuronide		0.85 ± 0.61	0.0105 ± 0.0082
2	21.0	581	287	Cy-3-sam	*3.17* ± 0.37	*3.11* ± 0.52	*0.1102* ± 0.0297
3	21.5	449	287	Cy-3-GLC	*13.42* ± 1.57	*4.54* ± 0.87	*0.0380* ± 0.0114
4	22.2	463	287	Cy monoglucuronide		T[e]	T
5	22.2	727	581/422/287	Cy-3-sam-5-rha	*24.16* ± 2.83	*18.38* ± 4.29	*0.0830* ± 0.0107
6	23.7	595	449/287	Cy-3-rut	*61.63* ± 7.22	*37.15* ± 5.75	*0.0662* ± 0.0064
7	25.4	433	271	pelargonidin-3-GLC	*0.29* ± 0.03	T	T
8	26.4	447	271	pelargonidin monoglucuronide		0.99 ± 0.21	0.0351 ± 0.0091
9	27.3	463	301	isopeonidin-3-GLC		0.60 ± 0.17	0.0052 ± 0.0020
10	27.9	463	301	peonidin-3-GLC		1.67 ± 0.23	0.0141 ± 0.0024
11	27.9	579	433/271	Pg-3-rut	*2.41* ± 0.28	*2.20* ± 0.65	*0.0996* ± 0.0235
12	28.7	477	301	peonidin monoglucuronide		3.51 ± 1.35	0.0303 ± 0.0111
13	28.9	609	463/301	isopeonidin-3-rutinoside		1.51 ± 0.30	0.0027 ± 0.0004
14	30.0	609	463/301	peonidin-3-rutinoside		1.77 ± 0.51	0.0032 ± 0.0006
				total	105.08 ± 12.31	76.25 ± 9.43	0.0728 ± 0.0077

[a] Values are means ± SEM; $n = 5$. [b] RT, retention time; parent ACNs present in BRB are in bold and italic. [c] Recoveries of metabolites were calculated based on their most likely parent compounds. Metabolites of Cy-3-GLC were assumed to include peaks 1, 4, 9, 10, and 12; Pg-3-rutin metabolite was assumed to be peak 8; Cy-3-rutin metabolites were assumed to include peaks 13 and 14. [d] T, trace.
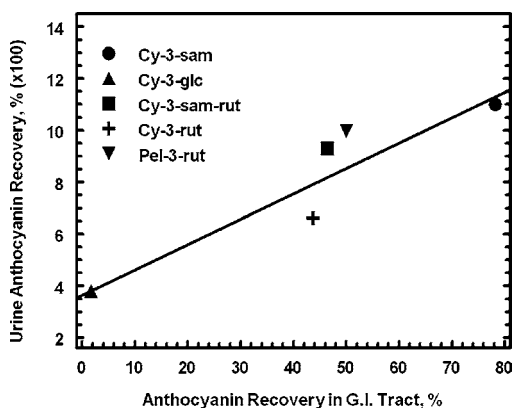
Figure 6. Relationship of recovery of ACNs in the GI tract with the recovery of the same ACN during a 4 h period following feeding of BRB. The *x*-axis is total ACN recovery in GI tract, and the *y*-axis represents urine ACN recovery (%, ×100).
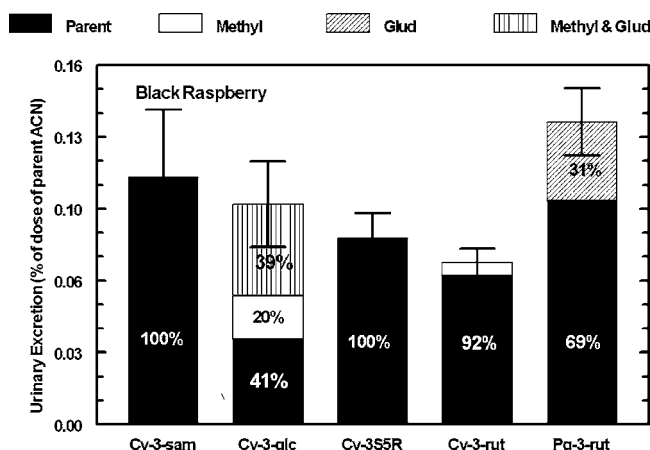
Figure 7. Urinary excretion of parent ACNs and their metabolites (methylated, glucuronidated, or methylated plus glucuronidated) as a percentage of the dose. The percentage of the total recovered for each form of the individual ACN is indicated inside the bar.

to the ileum, cecum, and colon. The fate of ACNs can significantly impact the TAC in the GI tract. He et al. (*40*) found that rats exposed to ACN rich extracts developed less and smaller aberrant crypt foci than rats on a control diet. They also found that the inhibition positively correlated to the ACN concentration in the feces across diets but was not correlated to the ACN concentrations in the urine. They suggested that the colon cancer preventive effect was related to the concentration of ACNs in the GI tract rather than the amount in the blood, probably due to direct absorption into colonic epithelial cells.

The proportion of the different ACNs in the different segments of the GI tract varies (**Figure 2**). Except for Cy-3-GLC, the profile of the four dominant ACNs in the GI contents throughout the whole small intestine is very similar to that in the berry. However, in the cecum and colon, the profiles of the ACNs in the GI contents exhibited large differences in relative amounts as compared to what was in BRB. Cy-3-GLC was not detected in the three segments of the small intestine but appeared in the cecum and colon where Cy-3-sam was the major ACN and Cy-3-sam-5-rha and Cy-3-rut were present in lesser amounts (**Figure 3**). One possible explanation of the high recovery (the recovery of Cy-3-sam was higher than 100% in one animal) is that Cy-3-sam-5-rha, the second most abundant ACN in BRB, may have been deglycosylated by intestinal organisms to Cy-3-sam. Biotransformation of Cy di- or triglycosides by gut microflora to Cy-3-GLC found in the cecum and colon has been reported previously (*23*). Cy-3-rut was also found to form Cy-3-GLC through cleavage of rhamnose by fecal microflora, and Cy-3-rut was degraded more slowly than Cy-3-GLC in the presence of 5% fecal slurry containing active gut microflora (*23*). α,L-Rhamnosidase activity was present in human fecal suspensions (*23*). He et al. (*40*) found that losses in the intestinal contents of rats were high for ACN GLCs, moderate for galactosides, and negligible for arabinosides or xylosides. This means that for the same aglycone, the sugar moiety is an important factor in determining their concentration in different segments of the GI tract. Possible reasons for these differences may be in their stability, differences in absorption in the stomach and/or small intestine (*41*), and other factors. It is clear that the environment in different segments of GI tract is critical to the stability and may further determine the forms that exist in the GI tract and that are absorbed and/or metabolized.

ACN Absorption vs Degradation. The virtual complete disappearance of Cy-3-GLC from the small intestine after the meal is surprising. Although the disappearance of Cy-3-GLC in the entire GI tract was 98%, the total recovery of Cy-3-GLC and its possible metabolites in the urine did not show a

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corresponding significant increase as compared to that of other parent ACNs. Data in **Figure 6** furthermore indicate that higher recovery of ACNs in the gut was associated with increased recovery in the urine. This likely indicates that the disappearance of Cy-3-GLC in the small intestine was caused by degradation rather than absorption and movement into the circulation. However, the ratio of the ACN di- or triglycosides did not change (**Figure 3**) within the different segments of the small intestine. The significant increase of Cy-3-sam in the cecum and colon may be the result of deglycosylation of Cy-3-sam-5-rha because the rhamnose has been found to be cleaved more easily than other sugars.

In previous studies (*8*, *24*), ACNs with more complex sugar conjugates were not cleared from the blood as rapidly as compared to cyanidin-3-GLC. The ratios for the area under the plasma ACN curve, adjusted for amount consumed, was 1:2:6.2 for Cy-3-GLC:Cy-3-rut:Cy-3-sam. Similar ratios for the parent ACNs in the urine in this study (**Table 1**) were as follows: 1:1.7:2.9 for Cy-3-GLC:Cy-3-rut:Cy-3-sam. Thus, the relative amounts in the GI tract are reflected in plasma and urine. This suggests that degradation of ACNs within the GI tract may be a limiting factor for their absorption and if the ACNs are more stable within the GI tract, greater quantities can be absorbed. One might expect the converse with low recovery in the GI tract associated with increased absorption and increased amounts found in the urine. However, one must realize that urinary excretion cannot be used strictly as a measure of absorption as numerous transformations can occur during the absorption process. Excretion of ACNs in the bile, which is another possible route of disposition, was not measured in this study.

ACN Methylation and Conjugation. Absorption and metabolism of BRB ACNs in the pig were found to have a similar metabolic pattern to that observed in our previous studies with other berries (*7*, *8*, *24*). Metabolism of Cy-3-GLC was by methylation (20%), glucuronidation, or a combination of methylation and glucuronidation (39%). The total of all of the apparent metabolites of Cy-3-GLC was greater than Cy-3-GLC in the urine (**Table 1** and **Figure 7**). Only methylated forms (8%) were found as metabolites of Cy-3-rut, and only the glucuronidated metabolite was found from Pg-3-rut (**Figure 7**), which was similar to our previous results (*8*, *24*), The amounts of the parent compounds of the diglycosides of Cy and Pg in the urine were greater than their respective metabolites (**Figure 7**). However, the amount of Pg-3-rut metabolites recovered in the urine (31%) was less than we observed following a meal of Marion blackberry in which over 91% of the Pg in the urine was in the form of Pg monoglucuronide.

Remarkably, no other metabolites that had an absorbance at 520 nm other than the deglycosylated products and trace amounts of Cy in the cecum and colon were found. This means that as major metabolites of ACNs, methylated and glucuronidated conjugates were largely formed in body tissues and not secreted back into the GI tract. Even though we did not observe any anthocyanidins in urine or plasma, they might be formed in the epithelial cells of the intestine and serve as substrates for phase II enzymes to form the different conjugated metabolites either in the intestinal epithelial cells or in the liver.

Conclusions. The antioxidant capacity and concentration of ACNs within the GI contents paralleled each other throughout the GI tract of the weanling pig 4 h after a meal of BRB. Nearly 98% of Cy-3-GLC in GI tract disappearred, but only 22% of the Cy-3-sam and 50−56% of Cy-3-sam-5-rha, Cy-3-rut, and Pg-3-rut disappearred. Upon the basis of urinary excretion, the

presence of complex sugars (sambubiose and rutinose) on Cy largely prevented its methylation or glucuronidation although a small amount of methylation of Cy-3-rut was observed. Of the total Cy-3-sam, Cy-3-sam-5-rha, and Cy-3-rut recovered in the urine, 92−100% was as the parent compound whereas only 41% of the total of Cy-3-GLC recovered in the urine was as the parent compound. Pg was metabolized only to the monoglucuronide and was not methylated. Clearly, the ACN aglycone and the glycoside have major effects on the absorption and/or metabolism of ACNS.

ABBREVIATIONS USED

ACN, anthocyanin; BRB, black raspberry; BW, body weight; Cy, cyanidin; GI, gastrointestinal; GLC, glucoside; HPLC, high-performance liquid chromatography; MS, mass spectrometer; *m/z*, mass-to-charge ratio; ORAC$_{FL}$, oxygen radical absorbance capacity with fluorescein as fluorescent probe; Pg, pelargonidin; rha, rhamnoside; rut, rutinoside; sam, sambubioside; SPE, solid phase extraction; TAC, total antioxidant capacity; TFA, trifluoroacetic acid.

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