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Anthocyanin Metabolism in Rats and Their Distribution to Digestive Area, Kidney, and Brain

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Anthocyanins are present in human diet due to their wide occurrence in fruits and beverages. They possess antioxidant activities and could be involved in several health effects. The aim of this study was to investigate anthocyanin metabolism and distribution in the digestive area organs (stomach, jejunum and liver) and kidney, as well as a target tissue (brain) in rats fed with a blackberry (*Rubus fruticosus* L.) anthocyanin-enriched diet for 15 days. Identification and quantification of anthocyanin metabolites was carried out by HPLC−ESI-MS−MS and HPLC−DAD, respectively. The stomach exhibited only native blackberry anthocyanins (cyanidin 3-*O*-glucoside and cyanidin 3-*O*-pentose), while in other organs (jejunum, liver, and kidney) native and methylated anthocyanins as well as conjugated anthocyanidins (cyanidin and peonidin monoglucuronides) were identified. Proportions of anthocyanin derivatives differed according to the organ considered, with the liver presenting the highest proportion of methylated forms. Jejunum and plasma also contained aglycone forms. In the brain, total anthocyanin content (blackberry anthocyanins and peonidin 3-*O*-glucoside) reached 0.25 ± 0.05 nmol/g of tissue ($n = 6$). The urinary excretion of total anthocyanins was low (0.19 ± 0.02% of the ingested amount). Thus, organs of the digestive area indicated a metabolic pathway of anthocyanins with enzymatic conversions (methylation and/or glucurono-conjugation). Moreover, following consumption of an anthocyanin-rich diet, anthocyanins enter the brain.

KEYWORDS: anthocyanins; brain; blackberry; bioavailability; glucuronides; metabolism; rats

INTRODUCTION

Anthocyanins, which belong to the flavonoid family, are natural pigments widely distributed in fruits (*1*) and especially in berries. Their estimated daily intake (180 to 215 mg/day in the United States) is higher than that of other flavonoids (*2*). Anthocyanins are implicated in many biological activities (*3*) that may impact positively on health. These pigments may reduce the risk of coronary heart disease through modulation of arterial vasomotion (*4*), inhibition of platelet aggregation (*5*), or endothelial protection (*6*). In addition, anthocyanins could exert anticarcinogenic activities in vitro (*7*), reduce inflammatory insult (*6*), and also modulate immune response (*8*). All these effects might be mediated by their antioxidant activity (*9*, *10*).

Activities of anthocyanins are related to their metabolism. Anthocyanins are rapidly absorbed from both stomach (*11*, *12*) and small intestine (*13*). They then appear in blood circulation and urine as intact, methylated, glucurono- and/or sulfoconjugated forms (*14*−*16*). However, the distribution and metabolism of anthocyanins in tissues have not been well characterized. We have thus evaluated the anthocyanin concentrations in the digestive system organs (stomach, jejunum, and liver) and in excretory tissue (kidney) in rats fed a blackberry extract-enriched diet. This study was carried out with a blackberry anthocyanin extract because blackberry is characterized by one major pigment, cyanidin 3-*O*-glucoside.

Since recent studies have reported protective effects of polyphenols against neuronal deficits (*17*−*19*), we also focused our attention to the brain. Several animal investigations proposed a role for polyphenol-rich fruits, and particularly blueberry, in the prevention of age-related declines (*18*−*20*). Anthocyanins that are the main blueberry flavonoids (*21*) may exert protective effects against the oxidative damages implicated in age-related neuronal deficits (*22*, *23*). However, there is little data on the potential ability of anthocyanins to reach the brain. Therefore, we have investigated the capacity of anthocyanins to enter the brain in rats.

MATERIALS AND METHODS

Chemicals. Cyanidin 3-*O*-glucoside, peonidin 3-*O*-glucoside, cyanidin 3,5-*O*-diglucoside, cyanidin, and peonidin (**Figure 1**) were pur-

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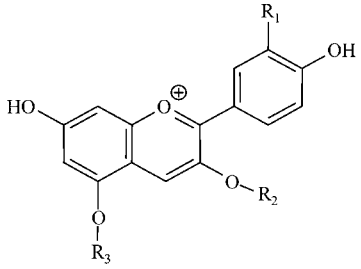

	R_1	R_2	R_3
Cyanidin	OH	H	H
Cyanidin 3-glucoside	OH	Glucose	H
Cyanidin 3-pentose	OH	Pentose	H
Cyanidin 3,5-diglucoside	OH	Glucose	Glucose
Peonidin	OCH_3	H	H
Peonidin 3-glucoside	OCH_3	Glucose	H
Peonidin 3-pentose	OCH_3	Pentose	H

Figure 1. Structures of anthocyanins and anthocyanidins.

chased from Extrasynthèse (Genay, France). Blackberry anthocyanin extract was supplied by Ferlux Mediolanum (Cournon d'Auvergne, France).

Animals and Diets. Twelve male Wistar rats, (Iffa-Credo, L'Arbresle, France) weighing approximately 250 g were housed two per cage in temperature-controlled rooms (22 °C) with a dark period from 8:00 to 20:00 h and access to food from 8:00 to 16:00 h. They were fed a semi-purified control diet (755 g/kg wheat starch, 150 g/kg casein, 50 g/kg peanut oil, 35 g/kg AIN-93M mineral mixture, 10 g/kg AIN-76A vitamin mixture) for one week (*24*). They were then randomly divided into two groups (each group comprising six rats) and individually housed in metabolic cages fitted with urine/feces separators. They received for 15 days (25 g diet/rat/day) either the control diet (control rats) or the control diet supplemented with 15 g blackberry extract per kg diet (i.e. 14.8 mmol anthocyanins per kg diet) (anthocyanin-fed rats).

All animals were maintained and handled according to the recommendations of the Institutional Ethics Committee (INRA), in accordance with decree No. 87-848.

Sampling Procedure. The day before sacrifice, urine was collected during 24 h in tubes containing 1 mL of 3 mol/L HCl, and exact food consumption was checked. Rats were sacrificed at 3 h after the beginning of the last meal (i.e., at 11:00 h) after being anesthetized with sodium pentobarbital (40 mg/kg body weight). At this time, rats have eaten ~60% of the amount of anthocyanins they were fed daily. All the blood (8−10 mL) was withdrawn from the abdominal aorta into heparinized tubes, and urine was collected in the bladder. Blood samples were centrifuged at 12000*g* for 5 min, and the plasma was quickly removed. Plasma and urine samples were rapidly acidified with 240 mmol/L HCl and stored at −20 °C until analysis. Next, the liver and kidneys were excised after being perfused with 20 mL of phosphate buffer saline to clean up the tissue. The stomach and jejunum were excised, and to remove any anthocyanins that adhered to the surface of the mucosa, they were washed with physiological solution until this solution was colorless. The brain was removed from bloodless rats. All tissue samples were rapidly frozen in liquid nitrogen and stored at −80 °C until analysis.

Sample Preparation. Urine samples were centrifuged at 12000*g* for 5 min, and the supernatant (20 μL) was injected and analyzed by HPLC as described below.

Anthocyanins present in plasma samples were extracted with a Sep-Pak C_{18} Plus solid-phase extraction cartridge (Waters, Milford, MA), using cyanidin 3,5-diglucoside as the internal standard as described previously (*12*) and then analyzed by HPLC (100 μL).

Extraction of anthocyanins from stomach and jejunum was carried out as follows: methanol containing 1% HCl was added to the frozen organs (9 mL per g of tissue). The samples were crushed using a homogenizer (Polytron) and centrifuged (3250*g*, 8 min, 4 °C). Supernatants were collected, and pellets were reextracted with 1% HCl in methanol (4 mL per g of tissue). The two methanolic supernatants were pooled and diluted 2-fold with 1% HCl aqueous solution for HPLC analysis (20 μL). The extraction recovery of anthocyanins was checked by adding cyanidin 3-glucoside to control tissues before crushing. Under these conditions, cyanidin 3-glucoside recovery from the stomach and jejunum was 93.7 ± 1.7% and 97.4 ± 1.9%, respectively.

A liver sample (1 g) and whole kidney were crushed in methanol containing 1% HCl (9 mL per g of tissue), spiked with 1.60 nmol cyanidin 3,5-diglucoside as internal standard, and centrifuged as described above. Use of this internal standard allowed correction for the loss of anthocyanins during sample preparation. The two methanolic supernatants were mixed and evaporated to dryness using a rotary evaporator at room temperature under reduced pressure. Dried tissue extracts were dissolved with 400 μL of 1% HCl aqueous solution. Once centrifugation was completed (12000*g*, 5 min), an aliquot (liver: 100 μL, kidney: 60 μL) was immediately analyzed by HPLC. The internal standard recovery was 28.3 ± 7.3% and 38.6 ± 7.6% in the liver and kidney, respectively.

Whole brains were crushed in 1% HCl aqueous solution (9 mL per g of tissue) and spiked with 1.60 nmol cyanidin 3,5-diglucoside as internal standard. After centrifugation, pellets were reextracted with 1% HCl aqueous solution (4 mL per g of tissue). The two aqueous supernatants were pooled, and anthocyanins were purified with a Sep-Pak C_{18} Plus solid-phase extraction cartridge (Waters, Milford, MA) (*12*) and analyzed by HPLC (100 μL) as described below. Internal standard recovery was 37.8 ± 13.7%.

We have verified in various control samples (tissues, plasma, urine) spiked with cyanidin 3,5-diglucoside or cyanidin 3-glucoside that neither HCl nor evaporating to dryness under reduced pressure at room-temperature broke down glycosides to aglycones during HPLC sample preparation. Moreover, cyanidin 3,5-diglucoside used as internal standard was not degraded into cyanidin 3-glucoside.

HPLC Analysis. Quantification of anthocyanins was performed by HPLC using a DAD 200 photodiode array detector (Perkin-Elmer, Courtabœuf, France) and a 785A UV−visible detector (Perkin-Elmer) at 524 nm. Samples were loaded onto a 150 × 4.6 mm i.d., 5-μm, Hypersil C18 column protected by a 10 × 4 mm i.d., 5 μm, Hypersil C18 guard column (Interchim, Montluçon, France). Elution was performed using water−H_3PO_4 (99:1) as solvent A and acetonitrile as solvent B at a flow rate of 1.0 mL/min. Analyses were carried out with linear gradient conditions from 100% A to 90% A for 10 min and then to 75% A for 30 min. Anthocyanin quantification was expressed as cyanidin 3-glucoside equivalents.

Identification of blackberry extract anthocyanins and anthocyanin metabolites was carried out by HPLC−ESI−MS−MS analysis. These analyses were performed on a Hewlett-Packard HPLC system equipped with API 2000 MS−MS detection (Applied Biosystem, Les Ulis, France), as previously described (*15*). The MS data were collected in multiple reaction monitoring mode by monitoring the transition of parent and product ions specific for each compound using a dwell time of 0.5 s. Anthocyanins were detected according to the respective *m*/*z* values of their parent and product ions: cyanidin 3-glucoside (449/287), cyanidin pentose (419/287), cyanidin malonylglucoside (535/287), cyanidin dioxalylglucoside (593/287), peonidin 3-glucoside (463/301), peonidin pentose (433/301), cyanidin (287/137), peonidin (301/201), cyanidin monoglucuronide (463/287), and peonidin monoglucuronide (477/301). An *m*/*z* value of 176 for the substitution group indicated a glucuronide residue. However, the exact site of glucuronidation could not be identified.

RESULTS

The HPLC profile of blackberry extract showed four peaks (**Figure 2**A). This anthocyanin extract contained approximately 47.9% anthocyanins. Their identification was performed by HPLC−ESI−MS−MS. Cyanidin 3-glucoside (peak 1) was the major pigment (91.2%). Peak 4 was identified as a cyanidin 3-pentose by detection of the respective parent and product ion pairs (*m*/*z* values 419/287) and accounted for 5% of total

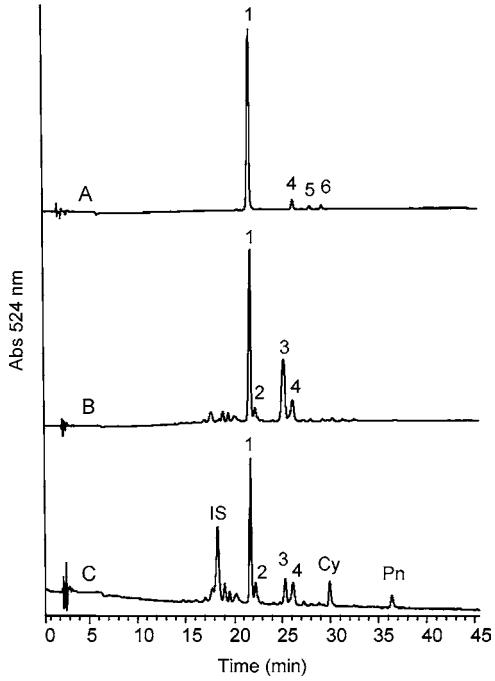

Figure 2. Representative HPLC chromatograms detected at 524 nm. (**A**) Blackberry anthocyanin extract; the composition is as follows: 1, cyanidin 3-glucoside; 4, cyanidin 3-pentose; 5, cyanidin 3-malonylglucoside; 6, cyanidin 3-dioxalylglucoside. (**B**) Urine and (**C**) plasma from rats fed a blackberry extract-supplemented diet; 2, cyanidin monoglucuronide; 3, peonidin 3-glucoside + peonidin monoglucuronide. Abbreviations: Cy, cyanidin; Pn, peonidin; IS, Internal Standard cyanidin 3,5-diglucoside.

anthocyanins. The two other minor components (peaks 5 and 6) were identified as acylated derivatives of cyanidin 3-glucoside in full scan mode over a mass range of *m*/*z* from 500 to 600. Their response to the specific cyanidin malonylglucoside transition (*m*/*z* values: 535/287) and cyanidin dioxalylglucoside transition (*m*/*z* values: 593/287), respectively, confirmed their structure. Therefore, the blackberry extract contained only derivatives of cyanidin.

Whereas no anthocyanins were detected in control urine, many peaks appeared in the urine of the anthocyanin-fed rats. Urine collected in the bladder and 24-h urine presented the same HPLC profile. The urinary HPLC profile (**Figure 2B**) showed anthocyanins from the blackberry extract as well as methylated and glucurono-conjugated derivatives. The presence of blackberry anthocyanins, cyanidin 3-glucoside (peak 1) and cyanidin 3-pentose (peak 4) was confirmed by detection of their specific parent and product ions (*m*/*z* values: 449/287 and 419/287, respectively). The two acylated derivatives of cyanidin 3-glucoside were also present in low concentrations. Peak 3 was identified as peonidin 3-glucoside according to its parent and product ion pair (463/301). Peonidin 3-pentose was also detected in urine by its specific transition (*m*/*z* values: 433/301). HPLC−ESI−MS−MS was able to identify glucurono-conjugated anthocyanidins. Peak 2 was characterized as a cyanidin monoglucuronide (463/287), and a peonidin monoglucuronide (477/301) was detected in peak 3. Thus, peak 3 contained both a glucoside and a glucuronide of peonidin and represented 26% of the urinary excreted forms. The mean urinary excretion of anthocyanins over a 24-h period was estimated by taking all peaks into account. Urinary excretion of native anthocyanins plus metabolites detected at 524 nm was 0.62 ± 0.08 μmol/24 h (for six rats). The blackberry anthocyanin-supplemented diet contained 14.8 mmol anthocyanins per kg diet, and the amount of anthocyanins ingested on the day before sacrifice was 318 ± 7 μmol (n = 6). The total urinary excretion of anthocyanins accounted for 0.19 ± 0.02% (n = 6) of anthocyanin intake.

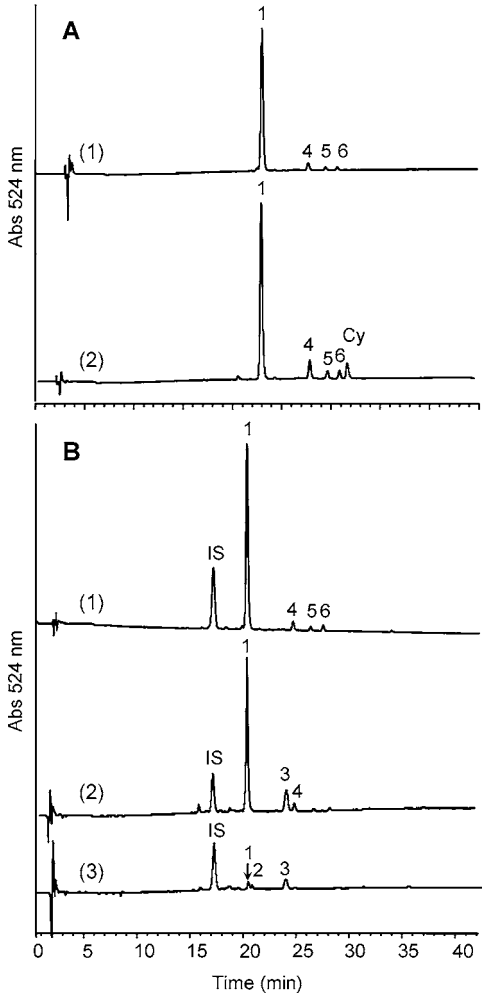

Figure 3. (**Panel A**) HPLC chromatograms of (1) stomach and (2) jejunum from rats fed a blackberry extract-supplemented diet. (**Panel B**) HPLC chromatograms of (1) brain, (2) kidney, and (3) liver from rats fed a blackberry extract-supplemented diet. Detection was performed at 524 nm. For peak identification, see the legend for **Figure 1**. IS: Internal Standard.

Whereas no anthocyanins were detected in plasma from control rats, the HPLC fingerprint of plasma from anthocyanin-fed rats revealed the presence of native anthocyanins. Several anthocyanin metabolites (**Figure 2C**) were also detected. HPLC−ESI−MS−MS identified methylated forms of cyanidin 3-glucoside and cyanidin 3-pentose (peonidin 3-glucoside and peonidin 3-pentose, respectively). Monoglucuronide derivatives (cyanidin monoglucuronide and peonidin monoglucuronide) were also specified. For the first time, aglycones such as cyanidin (287/137) and peonidin (301/201) were detected in plasma. Plasma anthocyanin concentration reached 0.36 ± 0.02 μmol/L (n = 6) at the time of sacrifice, i.e., 3 h after the beginning of the last meal. The proportion of cyanidin 3-glucoside and peonidin 3-glucoside + peonidin glucuronide relative to total plasma anthocyanins was 41.7% and 8.1%, respectively.

In the stomach from anthocyanin-fed rats, only blackberry anthocyanins were detected (cyanidin 3-glucoside, cyanidin 3-pentose, and the two minor acylated anthocyanins, **Figure 3A**-1). No metabolites were found. HPLC−ESI−MS−MS analysis of the jejunum revealed the presence of blackberry anthocyanins as well as traces of methylated forms (peonidin 3-glucoside, peonidin 3-pentose) and glucurono-conjugated derivatives (cyanidin monoglucuronide, peonidin monoglucu-

Table 1. Anthocyanin Content[a] in Tissues from Rats Fed for 15 Days with a Blackberry Anthocyanin-Enriched Diet[b]

tissue	Cy 3-glc concentration[c] (Cy 3-glc proportion relative to total anthocyanins, %)	total anthocyanin concentration
	nmol Cy 3-glc/ g of tissue	nmol Cy 3-glc equiv/ g of tissue
stomach	62.9 ± 5.4 (91.7%)	68.6 ± 5.8
jejunum	485 ± 54 (80.2%)	605 ± 71
kidney	2.16 ± 0.94 (66.1%)	3.27 ± 1.13
liver	0.05 ± 0.01 (13.2%)	0.38 ± 0.04
brain	0.21 ± 0.05 (84.0%)	0.25 ± 0.05
	nmol Cy 3-glc/ mL of plasma	nmol Cy 3-glc equiv/ mL of plasma
plasma	0.15 ± 0.02 (41.7%)	0.36 ± 0.02

[a] Values are expressed as means ± SEM of six rats. [b] The diet contained 14.8 mmol of anthocyanins, i.e., 13.5 mmol (91.2%) of Cy 3-glc per kg diet. [c] Cy 3-glc: cyanidin 3-*O*-glucoside.

ronide). Presence of cyanidin was also confirmed (**Figure 3A**-2). The jejunum was the most anthocyanin-rich tissue, with an anthocyanin content of 605 ± 71 nmol/g tissue ($n = 6$) at 3 h after the beginning of the last meal. In the liver (**Figure 3B**-3), native anthocyanins (cyanidin 3-glucoside, cyanidin 3-pentose), their methylated forms (peonidin 3-glucoside, peonidin 3-pentose), and slight amounts of their conjugated monoglucuronides (cyanidin glucuronide, peonidin glucuronide) were detected. Concentrations of peonidin 3-glucoside and peonidin glucuronide accounted for over 57% of total anthocyanins in the liver. The HPLC profile of anthocyanins in kidney is presented in **Figure 3B**-2. HPLC−ESI−MS−MS analysis allowed also identification of blackberry anthocyanins, methylated forms (peonidin 3-glucoside, peonidin 3-pentose), and traces of monoglucurono-conjugated forms of cyanidin and peonidin. Concentrations of peonidin 3-glucoside + peonidin glucuronide accounted for ∼21% of total kidney anthocyanins. HPLC analysis of brain from anthocyanin-fed rats (**Figure 3B**-1) exhibited blackberry anthocyanins. The presence of a methylated form (peonidin 3-glucoside) was also detected by HPLC−ESI−MS−MS. Specific transitions of cyanidin 3-glucoside, cyanidin 3-pentose, and peonidin 3-glucoside (*m*/*z* values: 449/287, 419/287, and 463/301, respectively) are given in **Figure 4**.

In all these tissues, all the compounds described above were specific to the anthocyanin diet since no peaks were detected in control samples. Total anthocyanin concentrations in various tissues and in plasma are given in **Table 1**. The kinds of metabolites found in the biological fluids and tissues are summarized in **Table 2**.

DISCUSSION

The aim of this study was to evaluate the distribution and metabolic fate of anthocyanins in several tissues to gain a better understanding of their health effects. Tissues from the digestive system (stomach, jejunum, liver), an excretion organ (kidney), as well as a target tissue (brain) were studied. We used a model that mimics a regular intake of anthocyanins: rats were fed for 15 days with an anthocyanin-enriched diet. The diet was supplemented with a blackberry extract since this anthocyanin source is characterized by one major pigment (>90%), cyanidin 3-glucoside. Moreover, this extract also contained a cyanidin 3-pentose that according to its chromatographic profile could certainly be cyanidin 3-xyloside, as previously reported in blackberry and chokeberry (*25*−*27*). HPLC−ESI−MS−MS

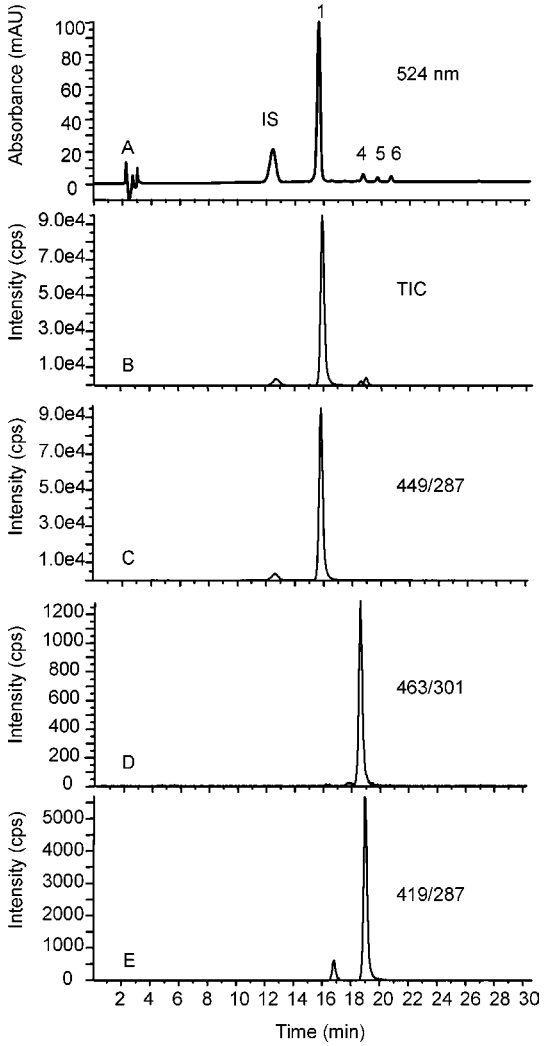

Figure 4. HPLC−ESI-MS−MS analysis of anthocyanin metabolites in brain from rats fed a blackberry extract-supplemented diet. (**A**) Detection at 524 nm. Detection of the respective *m*/*z* values of parent and product ions. (**B**), Total ionic current TIC; (C), cyanidin 3-glucoside; (D), peonidin 3-glucoside; (E), cyanidin 3-pentose. For peak identification, see the legend for **Figure 1**.

achieved identification of two minor anthocyanins as being acylated (cyanidin 3-malonylglucoside and cyanidin 3-dioxalylglucoside), according to previous studies on various blackberry genotypes (*27*, *28*). Therefore, the blackberry extract-enriched diet exclusively provided cyanidin glycosides.

The stomach seems to be an essential organ in anthocyanin absorption. Indeed, recent studies have demonstrated that anthocyanins could permeate the gastric wall (*11*, *12*). In the present study, only native blackberry anthocyanins were detected in the stomach, thus indicating that anthocyanins were absorbed across this organ as intact forms, as we have previously suggested (*12*). The mechanism of anthocyanin permeation in the stomach remains unknown, but recently Passamonti et al. (*29*) suggested that an organic anion carrier, bilitranslocase, expressed in the gastric epithelium could be involved in the absorption of anthocyanins at this level. The small intestine (particularly the jejunum) has been reported as a key site of flavonoid absorption (*30*). However, there are very few data on anthocyanin intestinal metabolism. In the present study, native blackberry anthocyanins as well as methylated, glucurono-conjugated forms and cyanidin were identified in the jejunum. Moreover, we have recently detected methylated and/

Table 2. Anthocyanin Metabolites[a,b] Found in Rats Fed for 15 Days with a Blackberry Anthocyanin-Enriched Diet

		biological fluid		tissue				
class of compounds	identified molecules	urine	plasma	stomach	jejunum	kidney	liver	brain
native forms	cyanidin 3-glucoside	+	+	+	+	+	+	+
	cyanidin 3-pentose	+	+	+	+	+	+	+
	cyanidin 3-malonylglucoside	+	+	+	+	+	-	+
	cyanidin 3-dioxalylglucoside	+	+	+	+	+	-	+
methylated forms	peonidin 3-glucoside	+	+	-	+	+	+	+
	peonidin 3-pentose	+	+	-	+	+	+	-
glucuronidated and/or methylated forms	cyanidin monoglucuronide	+	+	-	+	+	+	-
	peonidin monoglucuronide	+	+	-	+	+	+	-
aglycone forms	cyanidin	-	+	-	+	-	-	-
	peonidin	-	+	-	-	-	-	-

[a] + Presence of compound (detected by HPLC–ESI-MS–MS). [b] - Absence of detection of compound.

or glucuronidated derivatives in plasma following in situ intestinal perfusion of blackberry anthocyanins in rats (*13*). Intestinal mucosa contained various conjugation enzymes. The formation of methylated derivatives could result from the action of intestinal catechol *O*-methyl transferase, as described for various flavonoids (*31*, *32*). Furthermore, flavonoids are rapidly glucuronidated in the small intestine (*32*, *33*). By comparison with flavonoid metabolism, the presence of catechol *O*-methyl transferase and UDP-glucuronosyl transferase activities in the small intestine could explain the formation of methylated and glucurono-conjugated forms of anthocyanins at this level. The aglycone form (cyanidin) was also present in the jejunum according to Tsuda et al. (*34*). Deglycosylation by intestinal epithelial cells is a critical step in flavonoid metabolism (*35*). Hence, by analogy with other flavonoids, anthocyanins could be hydrolyzed by intestinal β-glucosidases (*36*, *37*), thus releasing the aglycone form. However, we could not evaluate the efficiency of β-glucosidase activity due to the instability of anthocyanin aglycones. This instability of anthocyanidins at physiological pH (*1*) could explain the low amount of cyanidin in the jejunum. The liver is a major site of enzymatic conversion, particularly methylation and glucuronidation (*38*, *39*). In agreement with previous studies (*34*, *40*), methylated forms were the main anthocyanins recovered in the liver. The presence of a high proportion of methylated anthocyanins in rat liver and the low amount of these derivatives in plasma suggested that these metabolites may be excreted from the liver directly into bile, as has previously been hypothesized (*12*, *13*). Moreover, traces of cyanidin and peonidin monoglucuronides were also detected in the liver. The kidney was the last organ where anthocyanins transited before urinary elimination. Methylated forms of anthocyanins were detected in the kidney, as previously reported (*34*, *41*). This organ has been described as one of the most important sites of methylation after the liver (*38*, *42*). On the other hand, we detected monoglucuronides of anthocyanidins in kidneys which could result from the action of a UDP-glucuronosyltransferase, since such an enzyme has been expressed in this organ (*39*). As we have previously shown (*13*, *24*), native anthocyanins as well as methylated and glucuronidated metabolites were present in urine, and their urinary excretion was low as compared to the amount ingested (*24*). Circulating forms detected in plasma (native anthocyanins, methylated and/or glucuronidated derivatives, and aglycones) provided evidence of anthocyanin metabolism in various tissues. Some methylated and glucuronidated derivatives have previously been identified in human urine and plasma (*14*−*16*). However, aglycones (cyanidin and peonidin) were not previously detected in plasma. They could correspond to intermediary metabolic forms before enzymatic conversion, i.e., glucuronidation.

Several reports have focused their attention on the potential protective effects of polyphenols from blueberries against the neuronal deficits associated with aging or neurodegenerative diseases (*18*, *19*, *23*). Since anthocyanins constitute a large part of blueberry polyphenols, they could be partly implicated in these effects. To understand the putative mechanisms underlying anthocyanin neuroprotection, it is important to establish whether anthocyanins are able to enter the central nervous system (CNS). This in vivo study is the first to demonstrate that anthocyanins target the brain of rats following consumption of an anthocyanin-enriched diet. Moreover, cyanidin 3-glucoside content was higher in the brain (0.21 ± 0.05 nmol/g of tissue) than in plasma (0.15 ± 0.02 nmol/mL), thus confirming that the presence of anthocyanins in the brain was not due only to residual anthocyanins in the vessels and/or the capillary endothelium. Furthermore, the excision of the brain was carried out in bloodless rats, thus minimizing the residual amount of blood in this organ. Anthocyanins detected in the brain homogenate were a reflection of their presence in the tissue. These molecules could permeate the blood−brain barrier, in accordance with a recent in vitro study showing that brain endothelial cell lines took up cyanidin 3-rutinoside and pelargonidin 3-glucoside (*43*). At this level, anthocyanins, known for their antioxidant properties (*44*), could exert protective activities against the oxidative damages responsible for numerous neurological disorders (*45*). Cyanidin 3-glucoside, which was the predominant form (84%) in the brain, has been shown to possess a high antioxidant capacity (*44*, *46*). Its presence at the cellular level could compensate the oxidative vulnerability of neuronal cells in aging or neurodegenerative diseases (*18*, *20*, *23*). However, the minimum anthocyanin concentrations needed to observe such effects are not known. Moreover, anthocyanins were rapidly distributed to the brain. Indeed, a preliminary test with a single oral dose of blackberry anthocyanins administered by gavage has shown that anthocyanins were present in the brain only 30 min after their administration (data not shown). However, the mechanisms of entry into the CNS remain unknown. A bilirubin-binding motif identified in bilitranslocase has been identified in the CNS (*47*). On the other hand, bilitranslocase was suggested to be involved in gastric anthocyanin absorption (*29*). Hence, it could be hypothesized that anthocyanin could enter the brain by means of a carrier similar to bilitranslocase.

In conclusion, this study highlighted an anthocyanin metabolism with enzymatic conversions (methylation and/or glucurono-conjugation) in the digestive area and indicated that native blackberry anthocyanins and their methylated forms reached a target organ (brain). A thorough knowledge of the identity of anthocyanin metabolites and of their tissue distribution is an essential step for testing their biological activities at cellular

and molecular levels. Future research will focus on the accessibility of anthocyanins to various organs in relation to their chemical structure, and we plan to investigate the kinetics of how anthocyanins reach them.

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