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Distribution and Excretion of Bilberry Anthocyanins in Mice

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The physiology and tissue distribution of bilberry anthocyanins were studied in mice. After oral administration of bilberry extract (100 mg/kg body weight), both unmodified and methylated anthocyanins appeared in the plasma. The plasma concentration of total anthocyanins reached a maximum of 1.18 $\pm$ 0.3 μM after 15 min and then sharply decreased. Their urinary excretion was highest between 0 and 6 h after administration and had ceased by 24 h. The total quantities of bilberry anthocyanins excreted into urine represented 1.88% (range, 0.62% to 2.45%) of consumed anthocyanins. Thirteen anthocyanins were identified in bilberry extracts. Of these, malvidin-3-glucoside and -3-galactoside were the principal anthocyanins in the plasma 60 min after administration. When mice were maintained for 2 weeks on a diet containing 0.5% of bilberry extracts, the plasma concentration of anthocyanins reached a maximum of 0.26 μM. Anthocyanins were detected only in the liver, kidney, testes, and lung, with maximum tissue concentrations of 605, 207, 149, and 116 pmol/g, respectively. In these organs, malvidin-3-glucoside and -3-galactoside were the predominant anthocyanins. Anthocyanins were not detectable in the spleen, thymus, heart, muscle, brain, white fat, or eyes. We conclude that bilberry anthocyanins were absorbed into the body and distributed in specific organs, particularly the liver, kidney, and testis. The most common anthocyanins in tissues were malvidin glycosides.

KEYWORDS: Bilberry anthocyanins; malvidin; liver; testes; mice

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INTRODUCTION

Anthocyanidins, which have a typical flavonoid structure (**Figure 1**), are important plant pigments responsible for red, blue, and purple colors. Their glycoside derivatives, anthocyanins, are widely distributed in colored fruits and vegetables, especially in berries (*1*−*5*). Anthocyanidins and anthocyanins have been shown to exhibit a range of biological effects, including antioxidant activity, anticarcinogenesis, induction of apoptosis, antiobesity, antidiabetes, and prevention of DNA damage (*6*−*14*). Interestingly, recent research suggested that anthocyanins can also prevent oxidative stress resulting from psychological stress (*15*). Consequently, the regular consumption of foods rich in anthocyanins has been considered to be associated with a reduced risk of developing chronic diseases (*16*, *17*).

Flavonoids are present in plant foods and their products, principally as glycosides (*3*). When consumed, flavonoids typically are hydrolyzed into their aglycone form by mucosal and bacterial enzymes in the alimentary canal. Subsequently, they are metabolized to the glucuronidated and/or sulfated derivatives via phase II reactions. Detectable quantities of flavonoid aglycones, such as quercetin, are absent in human blood (*18*−*20*). Hence, the glucuronidated and sulfated derivatives of flavonoids may be more appropriate metabolites for the evaluation of the beneficial effects of flavonoids under physiological conditions than their glycosides or parent aglycones. By contrast, anthocyanins were shown to be absorbed mainly in their native forms or after methylation (*14*, *21*−*23*). However, small quantities of glucuronide metabolites (*21*, *22*) and decomposition products such as protocatechuic acid were also detected (*24*). These observations highlight the fact that the metabolism of anthocyanins is quite different from that of other flavonoids (*25*).

Recently, a number of studies have reported protective effects of anthocyanins or of anthocyanin-rich products, following organ injury in mouse models, e.g., in the liver, kidney, and brain tissue (*15*, *26*, *27*). In order to evaluate the beneficial effects of anthocyanins, it is important to ascertain whether ingested anthocyanins are able to reach the target organs. However, detailed information concerning the tissue distribution of anthocyanins in mice is quite limited, especially in relation to daily consumption.

In this study, we therefore investigated the tissue distribution in mice of anthocyanins from bilberry extracts. We first evaluated the absorption ratio of bilberry anthocyanins after a single oral

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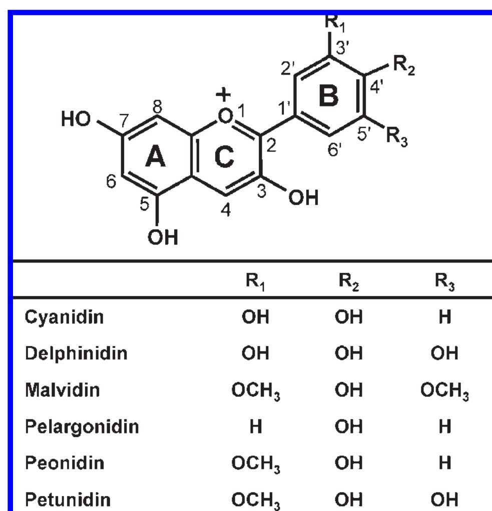

	R_1	R_2	R_3
Cyanidin	OH	OH	H
Delphinidin	OH	OH	OH
Malvidin	OCH_3	OH	OCH_3
Pelargonidin	H	OH	H
Peonidin	OCH_3	OH	H
Petunidin	OCH_3	OH	OH

Figure 1. Structures of anthocyanidins.

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administration. Subsequently, we determined the distribution of anthocyanins in a range of tissues (liver, kidneys, thymus, spleen, testes, heart, muscle, brain, white fat, and eyes) in mice that consumed a diet containing 0.5% bilberry extract. These data are discussed with respect to the physiological effects of bilberry anthocyanins and to the kinds of anthocyanins that are physiologically valuable.

MATERIALS AND METHODS

Materials. Bilberry extracts were extracted from fresh bilberries cultivated in Finland, using 80% ethanol acidified with hydrochloric acid. After evaporating to a powder, we analyzed anthocyanin contents as described below. Standards of the following anthocyanidins and anthocyanins were obtained from Extrasynthèse (Genay, France): delphinidin, delphinidin-3-glucoside, cyanidin, cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-rutinoside, petunidin, peonidin, peonidin-3-glucoside, pelargonidin, pelargonidin-3-glucoside, malvidin, malvidin-3-galactoside, and malvidin-3-glucoside. Trifluoroacetic acid (TFA) and ascorbic acid were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other reagents were of the highest grade available.

Animal Experiments. Male C57BL/6 mice (10 weeks; Japan SLC, Shizuoka, Japan) were housed in an air-conditioned room (23 ± 1 °C) under 12 h dark/12 h light cycles (light on 8:00−20:00) with free access to certified diet for mice (MF) from Oriental Yeast Co., Ltd. (Tokyo, Japan) and tap water. Animals were acclimated to these conditions for 10 days before use in experiments.

Protocol 1. After 12 h of starvation, we orally administered bilberry extracts dissolved in 10% citric acid to mice at a rate of 100 mg/kg body weight. Vehicle controls were given the same volume of 10% citric acid. The mice (5 mice per each group) were anesthetized with ether at individual time points (0, 15, 30, 60, and 120 min), and blood was collected from the abdominal vein using heparinized tubes (Capiject, Terumo Medical Corporation, Somerset, NJ). The plasma was separated by centrifugation at 800*g* for 10 min and acidified by the addition of 15 μL of formic acid to 1 mL of plasma. After the addition of 5 μL of 100 mM ascorbic acid, the plasma was stored at −80 °C for analysis.

Protocol 2. Bilberry extracts were administered orally to mice (500 mg/kg body weight; 5 mice per group) without prior starvation, and their urine was collected over four consecutive 6 h periods. The collection bottle, which was protected from light, contained 1 mL of 1% citric acid. The volume of urine was measured at each sample time, and the urine was stored at −80 °C.

Protocol 3. Bilberry extracts were blended into powdered MF diet at a rate of 0.5% by weight. After the acclimation period, the MF diet of a group of 10 mice was changed to the diet containing bilberry extracts and the mice maintained for a further two weeks. The control group consumed MF diet, which did not contain bilberry extracts. In order to accurately measure the quantity of food consumed, the diets were stocked in clean feeding equipment (Roden CAFE type M, Oriental Yeast Co., Ltd., Tokyo, Japan). The mice were sacrificed under anesthesia without starvation between 13:00 h and 15:00 h. Heparinized plasma was collected and treated as described above, and organs (liver, kidneys, thymus, spleen, testes, heart, muscle, brain, white fat, and eyes) were removed and weighed. Samples were stored immediately at −80 °C. Anthocyanins present in the samples were analyzed within one month because of their potential instability. This study was conducted according to the Guidelines for the care and use of laboratory animals of the University of Shizuoka.

Extraction of Anthocyanins from Plasma and Urine. The extraction procedure was as previously described (*28*) with some modifications. Each frozen, acidified plasma or urine sample was thawed at 37 °C in an e-ThermoBucket ETB (Taitec Co., Saitama, Japan). Aliquots (400 μL for plasma, 500 μL for urine) were loaded onto OASIS HLB (30 mg) extraction cartridges (Waters Co., Milford, MA), which were equilibrated with 0.01 M oxalic acid. After washing the cartridge with 2 mL of 0.01 mM oxalic acid, anthocyanins were eluted with 1 mL of methanol containing 0.5% TFA. The eluate was evaporated to dryness using a centrifugal concentrator (VC-96N, Taitec Co.). The residue was then dissolved in 150 μL of methanol containing 0.5% TFA, filtered with a 0.2 μm membrane filter (Millex-LG, Millipore Co., Bedford, MA), and analyzed by high performance liquid chromatography (HPLC) as described below.

Extraction of Anthocyanins from Mouse Tissues. Anthocyanins in individual tissues were extracted according to the method reported by Borges et al. (*29*) with some modifications. Tissue samples from three individual mice were combined for analysis except in the case of the liver. They were homogenized (Polytron PT-1300D, Kinematica Inc., Midfield, MA) in 10 volumes of 0.12 M perchloric acid containing 10 mM ascorbic acid and centrifuged at 3,000 rpm and 4 °C for 10 min. The supernatant was loaded on to an OASIS HLB (200 mg) extraction cartridge, which was equilibrated with 0.01 M oxalic acid. After the cartridge was washed with 10 mL of 0.01 mM oxalic acid, anthocyanins were eluted with 5 mL of methanol containing 0.5% TFA. The eluate was evaporated to dryness using a centrifugal concentrator. The residue was dissolved in 150 μL of methanol containing 0.5% TFA, centrifuged (14,000 rpm, 4 °C, 10 min), filtered (0.2 μm membrane filter), and analyzed by HPLC as described below.

HPLC-DAD. Anthocyanins were analyzed by HPLC in combination with a diode array detector (DAD) system, as described in our previous report (*5*). Briefly, the HPLC system employed to analyze anthocyanins was a JASCO system control program HSS-1500 (Tokyo, Japan) equipped with a JASCO-BORWIN chromatography data station, pump PU-1580, autosampler AS-1559, column oven CO-1565, and DAD system MD-1510 for monitoring at all wavelengths from 200−600 nm. The column, a Capcell Pak ACR (ϕ4.6 × 250 mm, S-5, 5 μm, Shiseido Co. Ltd., Tokyo, Japan), was used at 40 °C. Linear gradient elution was performed with solution A (0.5% TFA aqueous) and solution B (acetonitrile containing 0.1% TFA) delivered at a flow rate of 1.0 mL/min as follows: initially 92% of solution A; for the next 50 min, 85% A; for another 10 min, 70% A; for another 5 min, 40% A; and finally, 40% A for 10 min. The injected volume of the extract was 50 μL.

LC/MS/MS Analysis. Another set of plasma extracts dissolved in 200 μL of acetonitrile containing 0.1% formic acid was injected into the HPLC/Q-TOF-MS/MS system to obtain more information about which of the major anthocyanins were absorbed into the blood, malvidin glycosides or peonidin glycosides, or both. A portion of the filtrate (5 μL) was subjected to HPLC on a TSKgel ODS-100 V (3 μm, 1.0 × 150 mm, TOSOH, CO., Tokyo, Japan). The columns were maintained at 40 °C. Linear gradient elution was performed with solution A (0.1% formic acid) and solution B (acetonitrile with 0.1% formic acid) delivered at a flow rate of 20 μL/min as follows: initially, 90% of solution A and, for the next 20 min, 10% solution A. The eluate containing anthocyanins was introduced into the Q-TOF-MS/MS system (Type 6510, Agilent Technologies, Santa Clara, CA, US). The mass spectrometer was operated in the positive ion mode in the range *m*/*z* 250−600 using 3.5 kV for the detector voltage, 175 V for the fragmenter, 65 V for the skimmer, and at 325 °C gas temperature.

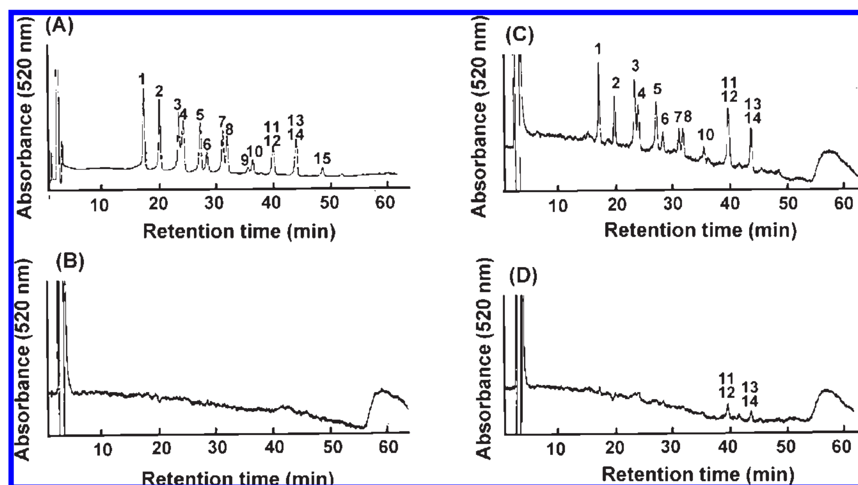

Figure 2. Typical HPLC profiles of berry extracts and plasma at 520 nm. (**A**) Bilberry extracts, (**B**) mouse plasma sample collected prior to the consumption of extract, (**C**) mouse plasma collected 15 min after an oral dose of bilberry extracts, and (**D**) mouse plasma collected 60 min after an oral dose. Peak numbers refer to **Table 1**.

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RESULTS AND DISCUSSION

Identification of Anthocyanins and Measurement of Concentrations in Bilberry Extracts. The bilberry extracts used in this study contained 15 kinds of anthocyanins as shown in **Figure 2A**. The retention times and spectra were compared with that of commercially available anthocyanidins and anthocyanins, and also compared with our previous data (*5*). Peaks that were identified as anthocyanins for which standards were unavailable were determined using calibration curves obtained from commercially available anthocyanins with the same aglycone structures. For example, delphinidin-3-glucoside was used for calculation of delphinidin-3-arabinoside content. Peaks 11 (malvidin-3-galactoside) and 12 (peonidin-3-glucoside) in **Figure 2A** had almost the same retention times and spectra under our HPLC conditions. Therefore, peaks which eluted at this point with a typical anthocyanin spectrum, were determined to be either peonidin-3-glucoside or malvidin-3-galactoside, and calculated using the calibration curve of malvidin-3-galactoside. Peaks 13 and 14 also gave almost identical retention times. The quantities of individual anthocyanins present in the bilberry extracts used in this study are summarized in **Table 1**. The total concentration was 67.3 μmol/100 mg extract.

Absorption Profiles of Anthocyanins in Plasma and Urine after Single Administration. The concentration profiles of total anthocyanins in mouse plasma after the consumption of 100 mg bilberry extracts/kg body weight (67.3 μmol anthocyanins/kg body weight) were investigated by HPLC-DAD. **Figure 2B–D** shows typical HPLC chromatograms at 520 nm for mouse plasma samples collected prior to the consumption of bilberry extract and 15 and 60 min after an oral dose of the extract, respectively. Fifteen minutes after administration, 13 peaks were detected in the plasma sample. The peak pattern was quite similar to that of the bilberry extracts administered (**Figure 2A**), indicating that, in these mice, bilberry anthocyanins were mainly absorbed into the body as intact anthocyanins. However, we could not find the detectable amounts of anthocyanins in plasma obtained from mice consuming the control diet (data not shown). These results paralleled those reported by Ichiyanagi et al. (*22*) for rats. The total concentration of anthocyanins in the plasma reached a maximum of approximately 1.2 μM at 15 min after administration and then sharply decreased almost to basal levels after 120 min (**Table 1**). Interestingly, peaks 11/12 and 13/14 still persisted at this time (**Figure 2D** and **Table 1**). These peaks corresponded to the overlapping peaks of malvidin and peonidin glycosides observed by HPLC, which were described above. Hence, we next identified the methoxy forms of these anthocyanins using the LC/MS/MS system. The selected ion mode analysis indicated that malvidin-3-galactoside and malvidin-3-glucoside (both m/z 493 > 331) were predominant in comparison to peonidin-3-glucoside (m/z 463 > 301) (data not shown). The half times for disappearance of individual anthocyanins in plasma are reported to be in the following order: delphinidin > cyanidin > petunidin = peonidin > malvidin (*22*), indicating that methoxy anthocyanins have properties that retain them in the body after absorption. Additionally, it was suggested that cyanidin- and delphinidin-glycosides are methylated and yielded peonidin- and malvidin-glycosides under physiological conditions (*14*, *22*, *23*). Therefore, our results indicate that malvidin glycosides might be the major anthocyanins present under physiological conditions, following the consumption of bilberry extracts, although peonidin was also present. Moreover, these methoxy anthocyanins may have been produced by the conversion of other absorbed anthocyanins.

We also investigated the urinary excretion of consumed anthocyanins in mice. After administration of 500 mg bilberry extract/kg body weight (336.5 μmol anthocyanins/kg) to mice, their urine was collected at 6 h intervals for 24 h, and urinary anthocyanins were analyzed by HPLC-DAD. Most of the anthocyanins were excreted into the urine between 0 and 6 h after administration. Only small quantities were excreted between 6 and 24 h except in one individual whose peak excretion occurred between 6 and 12 h (**Figure 3**). The average quantity of total anthocyanins excreted in the urine during 24 h after administration represented only 1.88% of the total anthocyanins ingested (range, 0.62%–3.62%). Detectable amounts of anthocyanins were not found in the control urine (data not shown). The administration of bilberry extracts in Protocol 2 (500 mg/kg body weight) was five times higher than that of Protocol 1, indicating that anthocyanins absorbed into the body may be excreted within 24 h, even if administration was quite high.

Tissue Distribution and Physiological Aspects of Bilberry Anthocyanins in Mice. As mentioned in the Introduction, there have been many studies of the physiological aspects of anthocyanins. In particular, information on their absorption profiles continues to be accumulated (*14*, *21–23*). However, detailed data on the distribution of anthocyanins in mice, as a result of their daily

Table 1. Time-Dependent Changes of Plasma Anthocyanins after the Administration of Bilberry Extracts[a]

peak no.		administration amounts (μmol/100 mg extracts/kg body weight)	nM: times after administration of bilberry extracts (min) 0	15	30	60	120
1	delphinidin-3-galactoside	10.2	u.d.	175 (154−211)	149 (115−214)	27 (u.d.-61)	u.d.
2	delphinidin-3-glucoside	8.9	u.d.	110 (94−133)	108 (53−170)	18 (u.d.-36)	6 (u.d.-17)
3	cyanidin-3-galactoside	8.3	u.d.	179 (156−219)	149 (98−138)	33 (nd-54)	u.d.
4	delphinidin-3-arabinoside	7.8	u.d.	114 (94−142)	110 (89−138)	24 (u.d.-50)	u.d.
5	cyanidin-3-glucoside	6.8	u.d.	128 (95−185)	124 (81−180)	19 (u.d.-57)	u.d.
6	petunidin-3-galactoside	2.4	u.d.	48 (42−53)	36 (19−60)	u.d.	u.d.
7	cyanidin-3-arabinoside	5.7	u.d.	45 (38−54)	45 (36−57)	17 (u.d.-28)	u.d.
8	petunidin-3-glucoside	4.6	u.d.	57 (52−64)	43 (u.d.-79)	u.d.	u.d.
9	peonidin-3-galactoside	0.8	u.d.	29 (14−55)	19 (u.d.-43)	u.d.	u.d.
10	petunidin-3-arabinoside	1.7	u.d.	21 (18−23)	10 (u.d.-29)	u.d.	u.d.
11,12	malvidin-3-galactoside /peonidin-3-glucoside	4.3	u.d.	145 (104−222)	113 (81−153)	52 (37−60)	8 (u.d.-25)
13,14	malvidin-3-glucoside /peonidin-3-arabinoside	4.7	u.d.	115 (101−140)	104 (76−137)	37 (27−47)	5 (u.d.-15)
15	malvidin-3-arabinoside	1.1	u.d.	9 (u.d.-14)	6 (u.d.-17)	u.d.	u.d.
total		67.3	u.d.	1,177 (1,000−1,514)	1,013 (762−1,481)	227 (135−386)	19 (u.d.-42)

[a] Values are indicated as the mean (min−max) of plasma anthocyanins. u.d., under the detection limit in this study (<15 nM).

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consumption in the diet, was lacking. As indicated above, anthocyanins present in bilberry extracts may be absorbed, either unchanged or as methoxy forms, during the first 15 min or so after their consumption, and then excreted into urine over the following 24 h. Therefore, our next focus was to examine the tissue distribution of anthocyanins during continuous consumption of bilberry extracts. In mice that consumed diets containing 0.5% bilberry anthocyanins for 2 weeks, body weight gain and food intake were similar to those in the control group during the experimental period (data not shown). The mean daily intake of bilberry extracts was 617.6 mg/kg body weight/day (415.6 μmol anthocyanins/kg body weight/day). Plasma and 11 organs (liver, kidney, testes, lung, spleen, thymus, heart, muscle, brain, white fat, and eyes) were sampled after 2 weeks on the diet, and their anthocyanin contents were investigated. As shown in **Table 2**, the mean total concentration of anthocyanins in plasma was 153 nM, ranging from under the detection limit (< 15 nM) to 258 nM. The predominant forms were methoxy anthocyanins, especially malvidin glycosides. Moreover, anthocyanins that occurred in the liver, kidney, testes, and lung were mainly malvidin glycosides. Our results are consistent with reports that after a single oral administration of anthocyanin-containing extracts, anthocyanins reached the liver, kidney, and lung in rats (*14*, *22*, *30*), although their absence from the liver and kidney has also been reported (*29*). Interestingly, malvidin glycosides were detected in the testes. As far as we know, this is the first report of anthocyanins localized in the testes of mice after daily consumption. Talavera et al. reported that anthocyanins could be localized in the brain when rats consumed a diet supplemented with 15 g blackberry extract per kg diet (i.e., 14.8 mmol anthocyanins per kg diet) for 15 days (*31*). Moreover, berry anthocyanins have been shown to improve cognitive brain functions and suppress stress-induced cerebral oxidation (*15*, *17*). These findings indicate that anthocyanin can be absorbed and then reach the brain, and may consequently regulate brain function. However, in this study, we were unable to find a detectable amount of anthocyanins in the brain from mice consuming 5 g bilberry extracts per kg diet (approximately 3.5 mmol anthocyanins per kg diet) for 14 days. If mice were given greater amounts of anthocyanins, we might have been able to detect anthocyanins in their brain. Anthocyanins were also not detectable in the spleen, thymus, heart, muscle, white fat, or eyes. The blood volume of a mouse (26 g body weight) is about 1.8 mL, of which half represents the plasma

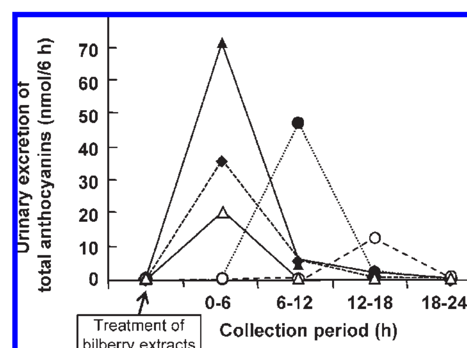

Figure 3. Time-dependent excretion in the urine of anthocyanins which were orally administered as a single dose of 500 mg bilberry extract/kg body weight (336.5 μmol anthocyanins/kg body weight). Analysis was performed by HPLC as described in Materials and Methods. The concentrations of all anthocyanins were calculated and summed. Each symbol represents an individual mouse.

Table 2. Anthocyanin Concentrations in Tissues of Mice Fed a 0.5% Bilberry Diet for 2 Weeks[a]

	pmol/g wet wt. of each tissue: Σ anthocyanins	Del-G[b]	Cy-G[b]	methoxy anthocyanins: Pet-G[b]	Mal-G/Peo-G[b]
plasma (nM)	153 (u.d.-258)	42 (u.d.-80)	26 (u.d.-80)	u.d.	84 (u.d.-178)
liver	173 (65−605)	34 (u.d.-178)	23 (u.d.-159)	8 (u.d.-49)	108 (42−218)
kidney	114 (u.d.-207)	25 (u.d.-62)	u.d.	u.d.	88 (u.d.-146)
testes	148.5	u.d.	u.d.	u.d.	148.5
lung	116.0	34.0	11.5	u.d.	70.5
spleen	u.d.				
thymus	u.d.				
heart	u.d.				
muscle	u.d.				
brain	u.d.				
white fat	u.d.				
eye balls	u.d.				

[a] Values are indicated as the mean (min−max) of 10 mice. u.d., under the detection limit in this study as follows: <15 nM in plasma, <10 pmol/g tissues. [b] Del-G, delphinidin glycosides (peak no. 1 + 2 + 4); Cy-G, cyanidin glycosides (peak no. 3 + 5 + 7); Pet-G, petunidin glycosides (peak no. 6 + 8 + 10); Mal-G/Peo-G, malvidin glycosides + peonidin glycosides (peak no. 9 + 11 + 12 + 13 + 14 + 15). Peak numbers refer to **Table 1**.

fraction. From calculations of the tissue distributions of anthocyanins within the body, it was estimated that 51.5% (range 36.4−100%) of anthocyanins within the body were localized in the liver, indicating that this organ may be the main target for the accumulation of absorbed anthocyanins.

It is recognized that the major physiological effects of anthocyanins result from their antioxidant properties. The antioxidant activities of flavonoids, including anthocyanins, are known to be related to the number and position of hydroxyl groups, particularly the *ortho*-dihydroxyl substitution at the 3′ and 4′ positions in the B-ring (*32*, *33*). In fact, their superoxide anion radical-scavenging activity and their inhibitory effect on hydrogen peroxide-induced lipid peroxidation have been reported to be stronger with more hydroxyl groups on the B-ring in the order delphinidin (3′,4′,5′-OH) > cyanidin (3′,4′-OH) > pelargonidin (4′-OH) (*34*). However, malvidin bears methoxy substitutions in the 3′ and 5′ positions. Hence, it might be supposed that the antioxidant potency of malvidin would be attenuated compared with that of other hydroxyl anthocyanins, such as delphinidin, cyanidin, and pelargonidin. However, malvidin has been reported to possess nearly the same antioxidant activity as that of other anthocyanins toward a range of reactive oxygen species, e.g., peroxynitrite, nitric oxide, and hydrogen peroxide (*35*,*36*). Moreover, malvidin is able to inhibit cAMP hydrolysis and consequently may regulate cell growth (*37*).

In conclusion, this study has shown that the most common anthocyanins in plasma and tissues were malvidin glycosides, although peonidin glycosides were also present. These and other anthocyanins are converted to methoxy derivatives during their absorption. During continuous consumption of bilberry extracts, anthocyanin levels are maintained in the liver, kidney, testes, and lung. This is the first report of anthocyanins distributed in the testes of mice after daily consumption. The liver appears to be the main target organ for absorbed anthocyanins. Future experiments will therefore focus on the strong antioxidant potency of the methoxy anthocyanin, malvidin, for example, in relation to possible amelioration of oxidative liver injury.

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