A Survey of Serum and Dietary Carotenoids in Captive Wild Animals[1]

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ABSTRACT Accumulation of carotenoids varies greatly among animal species and is not fully characterized. Circulating carotenoid concentration data in captive wild animals are limited and may be useful for their management. Serum carotenoid concentrations and dietary intakes were surveyed and the extent of accumulation categorized for 76 species of captive wild animals at Brookfield Zoo. Blood samples were obtained opportunistically from 275 individual animals immobilized for a variety of reasons; serum was analyzed for α- and β-carotene, lutein + zeaxanthin, lycopene, β-cryptoxanthin and canthaxanthin. Total carotenoid content of diets was calculated from tables and chemical analyses of commonly consumed dietary components. Diets were categorized as low, moderate or high in carotenoid content as were total serum carotenoid concentrations. Animals were classified as unknown, high, moderate or low (non-) accumulators of dietary cartenoids. Nonaccumulators had total serum carotenoid concentrations of 0–101 nmol/L, whereas accumulators had concentrations that ranged widely, from 225 to 35,351 nmol/L. Primates were uniquely distinguished by the widest range of type and concentration of carotenoids in their sera. Most were classified as high to moderate accumulators. Felids had high accumulation of β-carotene regardless of dietary intake, whereas a wide range of exotic birds accumulated only the xanthophylls, lutein + zeaxanthin, canthaxanthin or cryptoxanthin. The exotic ungulates, with the exception of the bovids, had negligible or nondetectable carotenoid serum concentrations despite moderate intakes. Bovids accumulated only β-carotene despite moderately high lutein + zeaxanthin intakes. Wild captive species demonstrated a wide variety of carotenoid accumulation patterns, which could be exploited to answer remaining questions concerning carotenoid metabolism and function. J. Nutr. 129: 380–390, 1999.

KEY WORDS: • *serum carotenoids* • *dietary carotenoids* • *animals* • *exotic animals*

The field of exotic animal nutrition is still in its infancy. Compared with humans and domestic animals, we know little about the nutrient requirements of captive wild animals. Better understanding of exotic animal nutrition is essential for their successful conservation and propagation. Schweigert (1995) suggested that research emphasis should be applied to studies concerning metabolism of both macro- and micronutrients because supplementation of these ingredients plays an important role in prevention of nutritional deficiencies in both free-ranging and captive wild species. In recent years, attention has focused on retinol and retinyl esters in exotic animals (Dierenfeld and Jessup 1990, Flurer and Schweigert 1990, Ghebremeskel et al. 1988, Ghebremeskel and Williams 1988, Schweigert 1987, Schweigert 1990). Information on circulating carotenoid concentrations in captive wild animals is meager. The available information is derived from select studies on a few species, mostly domestic and laboratory animals, which may not be applicable to exotic species.

Carotenoids may be important because of their role as vitamin A precursors and as free radical trapping agents. It has been suggested that dietary carotenoids influence longevity in humans as well as animals (Cutler 1984a and 1984b) and may be essential for proper immune function in humans and other species (Chew 1987, Chew et al. 1993). Carotenoids are known to influence sexual dichroism and protective coloration in fish (Goodwin 1986). Carotenoids accumulate in the plumage of birds; the color and patterns are important in avian biology for both sexual attraction and visual communication (Brush 1981). Like vitamin A, carotenoids are important for reproduction. β-Carotene was found to have a positive effect on fertility in cattle; its deficiency in cattle resulted in higher incidence of silent estrus, decreased conception rates, increased embryonic death and inferior composition of colostrum (Simpson and Chichester 1981).

Carotenoids may be classified into two main groups: very nonpolar $C_{40}H_{56}$ hydrocarbons called carotenes (including α-,β- and ξ-carotene and lycopene), and xanthophylls, which contain oxygen. Xanthophylls include zeaxanthin, lutein, cryptoxanthin and canthaxanthin.

Extensive species specificity exists with regard to the ability to utilize these carotenoids. Goodwin (1984) categorized groups of animals as accumulators, nonaccumulators and intermediate. Like humans, other primates are able to accumulate a wide range of carotenoid pigments (Krinsky et al. 1990, Mathews-Roth et al. 1990, Snodderly et al. 1990). Studies

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with humans also show considerable individual variability (Dimitrov et al. 1988), which may be true within other species. Nonaccumulators, including pigs, guinea pigs and rabbits, absorb negligible amounts of intact β-carotene and convert it largely to vitamin A (Beeson 1965, Bondi and Sklan 1984, Ganguly et al. 1953, Thompson et al. 1950). Rats are generally classified as nonaccumulators, but can actually accumulate β-carotene to a limited degree (Ganguly et al. 1953, Krinsky et al. 1990, Mathews-Roth et al. 1990, Shapiro et al. 1984). Early studies did not detect "carotene" (defined as a mix of α- and β-carotene, but predominantly β-carotene) in the blood of sheep or goats after a massive dose of carotene in the form of red palm oil or spinach puree (Goodwin and Gregory 1948). It was suggested that, like rabbits and pigs, they converted almost all absorbed β-carotene into vitamin A. The administration of large doses of carotene did not lead to elevated levels in the serum or liver of dogs (Frohring 1935, Steenbock et al. 1921). Domestic cats became vitamin A deficient when fed a vitamin A–free diet supplemented with either oral or intravenous β-carotene (Ahmad 1931, Gershoff et al. 1957), leading to the conclusion that cats do not convert it to vitamin A.

Between these two extremes fall the selective accumulators, animals able to accumulate only certain carotenoids. Birds accumulate xanthophylls in most tissues almost to the exception of all other carotenoids (Brush 1981, Goodwin 1984). Horses and certain breeds of cattle have the ability to absorb some β-carotene intact as well as convert it to vitamin A (Bondi and Sklan 1984, Parrish et al. 1947, Vander Noot et al. 1964).

The objective of this study was to examine and quantify the major serum carotenoids: lutein, β-cryptoxanthin, lycopene, α- and β-carotene and canthaxanthin in the widest possible representation of animals at Brookfield Zoo, Brookfield, IL, to serve as reference values for the management of captive exotic species. In addition, this study sought to characterize the usual dietary intake of carotenoids and from these data classify exotic species according to their level of accumulation.

MATERIALS AND METHODS

Animals. The animal collection at Brookfield Zoo consists of over 2500 animals including 150 species of mammals and 250 species of birds. Blood samples were obtained opportunistically from 76 different animal species (275 individuals) that were immobilized for any reason. Reasons included routine physicals, diagnosis of a specific ailment or just before killing by veterinary staff because of a poor health prognosis. Routine physicals were by far the most common reason. Animals were of both sexes and all ages.

Blood was drawn by veterinary staff into a tube with no anticoagulants added and protected from light. Upon return to the hospital, samples were centrifuged at 3700 × g for 7–10 min and serum separated. Serum tubes were wrapped in foil, labeled and frozen for up to 5 y at −80° C until thawed for analysis. Mean length of storage time was 1 y with most stored ≤2 y. The protocol was reviewed and approved by the Animal Care and Use Committees of both the University of Illinois at Chicago (UIC) and Brookfield Zoo.

Human samples. Concentrations of serum carotenoids in humans were measured in samples from 15 apparently healthy students and staff of the Department of Human Nutrition and Dietetics at UIC. Blood (10–30 mL) was obtained after an overnight fast. Serum was prepared, separated into 0.5-mL aliquots and stored at −80°C. This was the control serum that was analyzed in each run of carotenoid assay for quality control. Values represent a mean of 10 assays of the pooled serum.

Mammalian diets. *Marsupialia* (kangaroo). Low fiber herbivore pellet was the primary ingredient at 65% of the diet. It is an alfalfa-based pellet specially formulated to meet the nutrient needs of herbivorous animals and contains low levels of added β-carotene.

Apple, banana, sweet potato, carrot, spinach, lettuce, kale and celery made up 25% of the diet. The kangaroos had access to hay (<10% of the diet) in the winter. In the summer, they had access to and consumed plants in a grassy yard, which could increase the carotenoid content of the diet. This diet contained moderate (11–60 mg/kg dry matter) levels of carotenoids.

Primates (golden lion tamarin, callimico, capuchin monkey, spider monkey, spot-nosed guenon, sooty mangabey, mandrill, baboon, spectacled langur, colobus, siamang, orangutan, lowland gorilla). Primate biscuit, a grain-based pellet, comprised ~20% of the primate diet (ranging from 9% for the gorilla to 39% for the golden lion tamarin). Primate feeds are available in two forms, extruded biscuit or canned, and are specially formulated to meet the nutrient needs of nonhuman primates. For this reason, primate biscuits are available in several different types and may vary in protein content, fiber level and flavor.

Fruits, vegetables and leafy material comprise ~80% of the primate diet. Items may include any or all of the following: apple, grape, banana, orange, carrot, green bean, sweet potato, onion, celery, kale, lettuce, spinach, romaine, escarole and parsley.

Insects, nuts and seeds, and cooked eggs are possible treats and made up <1% of the total diet. All apes (siamang, orangutan, gorilla) received a multivitamin/mineral supplement daily. All pregnant primates received a prenatal vitamin supplement. All lactating primates received a multivitamin/mineral supplement daily. These diets were high (≥61 mg/kg dry matter) in carotenoids.

Cetacea (free-ranging and captive bottlenose dolphins). Dolphin diets were made up of various types of fish plus, in captivity, supplemental vitamin E and thiamin. Dolphins at Brookfield Zoo were offered herring, smelt, capelin and rainbow trout. Free ranging dolphins may consume many different types of fish as well as squid and crustaceans. These diets were classified as moderate in carotenoids due to the high carotenoid content of the trout.

Carnivora. 1) Canids and Ursids (cape hunting dog, wolf, spectacled bear, sloth bear, kodiak bear, polar bear). Cape hunting dogs were offered feline diet and wolves were offered a mix of feline diet and Canine diet (1:4) (both are specially formulated horsemeat-based mixes), plus bones in place of meat once weekly. Feline diet was extremely high in carotenoid content due to the high percentage of liver currently in the mix. Diets for cape hunting dogs were high in carotenoid content. The wolf diet was considered moderate in carotenoid content. Bears received dog nonpurified diet, canine diet, and, for the polar and kodiak bears, fish. These items comprised, on average, 50% of a bear's diet. The rest was made up of apple, carrot, sweet potato, lettuce and bread. The diets for the spectacled bear and the kodiak bear were considered medium in carotenoid content (36.4 and 28.2 mg β-carotene, respectively). Other bear diets were low (0–10 mg/kg dry matter) in carotenoids.

2)Mustelids and Viverrids (Asian small clawed otter, binturong). Otters received a specially formulated meat mix made at Brookfield Zoo. Primary ingredients included ground horsemeat, ground beef heart and ground nonpurified diet for cats. Rib bones and small fish were offered 1 d/wk. Binturongs were also offered a special meat mix. It consisted of the canine diet mentioned above combined with ground nonpurified diet for dogs and canned mixed vegetables. This was offered daily along with a small amount of steamed carrot and sweet potato. These diets were considered low in carotenoid content.

3) Felids. (A) Large cats (clouded leopard, snow leopard, Siberian tiger, jaguar, African Lion): feline diet, mentioned above, meets the nutrient requirements of cats. It comprised 70% of a cat's diet. They also received a small piece of chunk horsemeat daily, bones at least once weekly and a small piece of horse liver twice weekly. These diets were ranked high in carotenoid content due to the high concentrations of liver in the feline diet. (B) Small cats (sand cat, pallas cat, bobcat, caracal, fishing cat, ocelot, margay, Geoffroy's cat): Small cat diets consisted primarily of the canine diet (sand cat, two pallas cats, bobcats, one caracal, ocelot, Geoffrov's cat) mentioned above, or a similar beef-based feline diet (all other small cats). The beef-based feline diet was reported to contain <2% liver, whereas the canine diets were reported to contain no liver in the mix. Small cats did not receive additional liver as part of their diet. Most small cats were offered mice 1 d/wk. Pallas cats received mice daily; one animal received a rat because that was all he would consume (he was raised

TABLE 1

Serum carotenoid concentrations in Brookfield Zoo animals[1]

Common name, *Scientific name* (*n*)	Lutein + zeaxanthin	β-Cryptoxanthin	Lycopene	α-Carotene	β-Carotene	Other carotenoids
			nmol/L			
Mammals						
Marsupialia						
W. gray kangaroo, *Macropus fuliginosus melanops* (4)	–[2]	–	–	–	29 (0–69)	–
Primates						
Golden lion tamarin, *Leontopithecus rosalia rosalia* (2)	–	–	–	–	–	–
Callimico, *Callimico goeldii* (2)	49 (28–70)	–	–	–	52 (ND–104)	–
Capuchin monkey, *Cebus apella* (1)	49	–	–	–	–	–
Spider monkey, *Ateles geoffroyi* (2)	364 (346–382)	87 (72–101)	–	458 (298–618)	520 (248–792)	–
Spot-nosed guenon, *Cercopithecus ascanius schmidti* (5)	1310 (ND–2887)	47 (ND–123)	–	224 (ND–764)	465 (19–1695)	–
Sooty mangabey, *Cerocebus torquatus atys* (5)	1791 (1035–2120)	181 (127–242)	–	1543 (719–2425)	3237 (1773–4392)	–
Baboon, *Papio cynocephalus papio* (17)	301 (137–559)	76 (49–136)	–	56 (22–91)	59 (19–121)	–
Mandrill, *Papio sphinx* (5)	99.5 (44–139)	–	–	68 (24–188)	57 (22–134)	–
Spectacled langur, *Presbytis obscurus* (5)	550 (11–1282)	4 (ND–20)	–	40 (ND–86)	44 (ND–101)	–
Colobus monkey, *Colobus guereza* (4)	157 (ND–422)	4.2 (ND–25)	–	8.1 (ND–28)	56 (ND–171)	–
Siamang, *Hylobates syndactylus* (2)	266 (167–366)	–	–	34 (ND–67)	20 (ND–39)	–
Orangutan, *Pongp pygmaeus* (3)	1496 (951–2203)	195 (138–266)	58 (48–69)	379 (218–665)	681 (421–1064)	–
Lowland gorilla, *Gorilla gorilla gorilla* (7)	701 (172–2125)	11 (ND–74)	–	23 (ND–82)	22 (ND–65)	–
U.S. Human[3], *Homo sapiens* (15)	253	136	645	106	477	–
Cetacea						
Wild botttlenose dolphin, *Tursiops truncatus* (34)	11 (ND–21)	–	–	–	34 (ND–84)	–
Captive bottlenose dolphin (9)	–	–	–	–	–	–
Carnivora						
Grey wolf, *Canis lupus* (1)	–	–	–	–	–	–
Cape hunting dog, *Lycaon pictus pictus* (5)	–	–	–	–	–	–
Spectacled bear, *Tremarctos ornatus* (2)	–	–	–	–	–	–
Polar bear, *Ursus maritimus* (2)	–	–	–	–	–	–
Kodiak bear, *Ursus arctos middendorffi* (1)	–	–	–	–	–	–
Asian small-clawed otter, *Aonyx cinerea* (1)	–	–	–	–	–	–
Binturong, *Arctictis binturong* (2)	–	–	–	–	–	–
Sloth bear, *Melursus ursinus* (2)	6.2 (ND–12)	–	–	–	–	–
Sand cat, *Felis margarita* (1)	–	–	–	–	656	–
Pallas cat, *Felis manul* (9)	–	–	–	–	185 (ND–330)	–
Bobcat, *Felis rufus* (2)	–	–	–	–	143 (117–170)	–
Caracal, *Felis caracal* (2)	–	–	–	–	154 (ND–307)	–
Fishing cat, *Felis viverrinus* (2)	–	–	–	–	100 (97–102)	–
Ocelot, *Felis pardalis* (1)	–	–	–	–	661	–
Margay, *Felis wiedii* (4)	–	–	–	–	225 (52–427)	–
Geoffroy's cat, *Felis geoffroyi* (1)	–	–	–	–	540	–
Clouded leopard, *Panthera nebulosa* (3)	–	–	–	–	512 (384–652)	–
Snow leopard, *Panthera uncia* (5)	–	–	–	–	592 (358–853)	–
Siberian tiger, *Panthera tigris altaica* (3)	–	–	–	–	984 (773–1300)	–
Jaguar, *Panthera onca* (2)	–	–	–	–	1058 (838–1278)	–
African lion, *Panthera leo* (3)	–	–	–	–	479 (430–615)	–
Pinnipedia						
Sea lion, *Zalophus californianus* (5)	–	–	–	–	–	–
Walrus, *Odobenus rosmarus* (2)	–	–	–	–	–	–
Harbor seal, *Phoca vitulina* (6)	–	–	–	–	–	–
Proboscidae						
African elephant, *Loxodonta africana* (4)	–	–	–	–	–	–
Asian elephant, *Elephas maximus* (2)	–	–	–	–	–	–

TABLE 1 ***(continued)***

Serum carotenoid concentrations in Brookfield Zoo animals[1]

Common name, *Scientific name* (*n*)	Lutein + zeaxanthin	β-Cryptoxanthin	Lycopene	α-Carotene	β-Carotene	Other carotenoids
			nmol/L			
Hyracoidea						
Rock hyrax, *Procavia capensis* (2)	–	–	–	–	–	–
Perissodactyla						
Grant's zebra, *Equus burchelli bohmi* (5)	1.8 (ND–8.8)	–	–	–	233 (102–4068)	–
Grevy's zebra, *Equus grevyi* (2)	–	–	–	–	46 (15–84)	–
South American tapir, *Tapirus terrestris* (3)	–	–	–	12 (ND–21)	40 (ND–67)	–
Black rhino, *Diceros bicornis* (3)	–	–	–	–	–	–
Artiodactyla						
Warthog, *Phacochoerus aethiopicus* (1)	–	–	–	35	171	–
Pygmy hippo, *Choeropsis liberiensis* (1)	–	–	–	–	–	–
Bactrian camel, *Camelus bactrianus* (4)	–	–	–	–	–	–
Pere David's deer, *Elaphurus davidianus* (1)	–	–	–	–	–	–
Okapi, *Okapi johnstoni* (1)	–	–	–	–	–	–
Giraffe, *Giraffa camelopardis reticulata* (1)	–	–	–	–	–	–
Sitatunga, *Tragelaphus spekei* (1)	–	–	–	–	–	–
Greater kudu, *Tragelaphus strepsiceros* (1)	12	–	–	–	–	–
Eland, *Taurotragus oryx* (1)	–	–	–	–	–	–
Congo buffalo, *Syncerus caffer nanus* (1)	–	–	–	–	–	–
Banteng, *Bos javanicus* (1)	56	51	–	–	2073	–
Wisent, *Bison bonasus* (1)	76	52	–	26	2250	–
Addax, *Addax nasomaculatus* (2)	–	–	–	–	–	–
Klipspringer, *Oreotragus oreotragus* (1)	–	–	–	–	–	–
Siberian ibex, *Capra ibex sibirica* (9)	–	–	–	–	–	–
Birds						
Sphenisciformes						
Humboldt penguin, *Spheniscus humboldti* (15)	55 (18–116)	14 (ND–58)	–	–	–	–
Pelecaniformes						
Brown pelican, *Pelecanus occidentalis* (2)	651 (505–798)	–	–	–	–	–
Ciconiiformes						canthaxanthin
Scarlet ibis, *Eudocimus ruber* (2)	2313 (2087–2538)	17 (ND–34)	–	–	–	159 (115–204)
Sacred ibis, *Threskiornis aethiopicus* (3)	201 (72–311)	–	–	–	–	113 (69–150)
Hadada ibis, *Hagedashia hagedash* (1)	575	–	–	–	–	136
American flamingo, *Phoenicopterus ruber* (7)	–	–	–	–	–	24078 (16997–34347)
Greater flamingo, *Phoenicopterus roseus* (3)	–	–	–	–	–	35351 (28505–43554)
Anseriformes						canthaxanthin
Mandarin duck, *Aix galericulata* (3)	3230 (1264–5583)	140 (45–213)	–	–	–	333 (166–540)
Wood duck, *Aix sponsa* (1)	782	47	–	–	–	119
Hybrid teal, *Anas capensis x Amazonetta brasiliensis* (1)	775	–	–	–	–	89
Whistling duck, *Dendrocygna viduata* (2)	561 (490–631)	60 (54–65)	–	–	–	77 (58–96)
Brazilian teal, *Amazonetta brasiliensis* (1)	63	–	–	–	–	–
Charadriformes						
Gray gull, *Larus modestus* (7)	2478 (928–4599)	524 (31–1599)	–	–	–	–
Inca tern, *Larosterna inca* (7)	1253 (773–1573)	–	–	–	–	–

[1] Values are means (ranges).
[2] –, not detected (ND).
[3] Pooled serum from students and staff was analyzed for quality assurance in all assays. Values are means of 10 assays.

TABLE 2

Taxonomic classification by accumulation of dietary carotenoids

Species, *Scientific name* (*n*)	Total carotenoids[1]	Dietary carotenoid level[2]	Accumulation level[3]
	nmol/L		
Birds			
Greater flamingo, *Phoenicopterus roseus* (3)	35351 (28505–43554)	high	++
American flamingo, *Phoenicopterus ruber* (7)	24078 (16997–34347)	high	++
Mandarin duck, *Aix galericulata* (3)	3703 (1476–6337)	low	++
Grey gull, *Larus modestus* (7)	3001 (959–5150)	moderate	++
Scarlet ibis, *Eudocimus ruber* (2)	2489 (2290–2688)	low	++
Inca tern, *Larosterna inca* (7)	1253 (773–1573)	moderate	++
Wood duck, *Aix sponsa* (1)	948	low	++
Hybrid teal, *Anas capensis x Amozonetta brasiliensis*	864	low	++
Hadada ibis, *Hagedashia hagedash* (1)	711	low	++
Whistling duck, *Dendrocygna viduata* (2)	697 (603–792)	low	++
Brown pelican, *Pelecanus occidentalis* (2)	651 (504–798)	moderate	++
Sacred ibis, *Threskiornis aethiopicus* (3)	314 (192–462)	moderate	+
Humboldt penguin, *Spheniscus humboldti* (15)	69 (28.1–146)	moderate	+
Brazilian teal, *Amozonetta brasiliensis* (1)	63.3	low	+
Primates			
Sooty mangabey, *Cerocebus torquatus atys* (5)	6753 (4050–7970)	high	++
Orangutan, *Pongo pygmaeus* (3)	2810 (1810–3310)	high	++
Spot-nosed guenon, *Cercopithecus ascanius schmidti* (5)	2046 (383–5468)	high	++
Spider monkey, *Ateles geoffroyi* (2)	1429 (964–1893)	moderate	++
Lowland gorilla, *Gorilla gorilla gorilla* (7)	756 (172–2345)	high	+
Spectacled langur, *Presbytis obscurus* (5)	638 (10.6–1471)	high	+
Guinea baboon, *Papio cynocephalus papio* (17)	491 (295–864)	high	+
Siamang, *Hylobates syndactylus* (2)	319 (273–366)	moderate	+
Mandrill, *Papio sphinx* (5)	225 (91–461)	high	+
Colobus monkey, *Colobus guereza* (3)	226 (ND–647)	high	+
Callimico, *Callimico goeldii* (2)	101 (28–175)	moderate	0
Capuchin monkey, *Cebus apella* (1)	49	moderate	0
Golden lion tamarin, *Leontopithecus rosalia rosalia* (2)	–[4]	moderate	0
Artiodactyla			
Wisent, *Bison bonasus* (1)	2404	moderate	++
Banteng, *Bos javanicus* (1)	2180	moderate	++
Warthog, *Phacocherus aethiopicus* (1)	207	moderate	+
Greater kudu, *Tragelaphus strepsiceros* (1)	12	moderate	0
Pygmy hippo, *Choeropsis liberiensis* (1)	–	moderate	0
Bactrian camel, *Camelus bactrianus* (4)	–	moderate	0
Pere David's deer, *Elaphurus davidianus* (1)	–	moderate	0
Okapi, *Okapi johnstoni* (1)	–	moderate	0
Giraffe, *Giraffa camelopardis reticulata* (1)	–	moderate	0
Sitatunga, *Tragelaphus spekei* (1)	–	moderate	0
Eland, *Taurotragus oryx* (1)	–	moderate	0
Congo buffalo, *Syncerus caffer nanus* (1)	–	moderate	0
Addax, *Addax nasomaculatus* (2)	–	moderate	0
Klipspringer, *Oreotragus oreotragus* (1)	–	moderate	0
Siberian ibex, *Capra ibex sibirica* (9)	–	moderate	0
Carnivora			
Cats			
Siberian tiger, *Panthera tigris altaica* (3)	984 (773–1300)	high	++
Jaguar, *Panthera onca* (2)	1058 (838–1278)	high	++
Ocelot, *Felis pardalis* (1)	661	low	++
Sand cat, *Felis margarita* (1)	656	low	++
Snow leopard, *Panthera uncia* (5)	592 (357–853)	high	++
Geoffroys cat, *Felis geoffroyi* (1)	540	low	++
Clouded leopard, *Panther nebulosa* (3)	512 (383–652)	high	++
African lion, *Panthera leo* (3)	479 (430–615)	high	++
Margay, *Felis wiedii* (4)	225 (52–427)	low	++
Pallas cat, *Felis manul* (9)	185 (ND–330)	low	+
Caracal, *Felis caracal* (2)	154 (ND–307)	low	+
Bobcat, *Felis rufus* (2)	143 (117–170)	low	+
Fishing cat, *Felis viverrinus* (2)	100 (97–102)	low	+
Other Carnivores			
Sloth bear, *Melursus ursinus* (2)	6.2 (ND–12.3)	low	unk
Grey wolf, *Canis lupus* (1)	–	moderate	0
Cape hunting dog, *Lycaon pictus pictus* (5)	–	high	0
Spectacled bear, *Tremarctos ornatus* (2)	–	moderate	0
Polar bear, *Ursus maritimus* (2)	–	low	unk
Kodiak bear, *Ursus arctos middendorffi* (1)	–	moderate	0
Asian small-clawed otter, *Aonyx cinerea* (1)	–	low	unk
Binturong, *Arctictis binturong* (2)	–	low	unk

TABLE 2 *(continued)*

Taxonomic classification by accumulation of dietary carotenoids

Species *Scientific name* (*n*)	Total carotenoids[1]	Dietary carotenoid level[2]	Accumulation level[3]
	nmol/L		
Perissodactyla			
Grant's zebra, *Equus burchelli bohmi* (5)	233 (102–406)	moderate	+
Grevy's zebra, *Equus grevyi* (2)	46 (15–84)	moderate	+
South American tapir, *Tapirus terrestris* (3)	53 (ND–86)	moderate	+
Black rhinoceros, *Diceros bicornis* (3)	–	moderate	0
Cetacea			
Free-ranging dolphin, *Tursiops truncatus* (33)	44 (ND–98)	unk	unk
Captive dolphin, *T. truncatus* (9)	–	moderate	unk
Pinnipedia			
Sea lion, *Zalophus californianus* (5)	–	moderate	0
Walrus, *Odobenus rosmarus* (2)	–	moderate	0
Harbor seal, *Phoca vitulina* (6)	–	moderate	0
Marsupialia			
Western gray kangaroo, *Macropus fuliginisus melanops* (4)	29 (ND–69)	moderate	0
Proboscidae			
African elephant, *Loxodonta africana* (4)	–	moderate	0
Asian elephant, *Elephas maximus* (2)	–	moderate	0
Hyracoidea			
Rock hyrax, *Procavia capensis* (2)	–	high	0

1 Values are means (ranges).
2 High = ≥61 mg/kg dry matter; moderate = 11–60 mg/kg dry matter; low = 0–10 mg/kg dry matter.
3 ++ = high; + = moderate; 0 = low; unk = unknown.
4 –, not detected (ND).

on this and was on loan from Moscow Zoo). These diets were considered low in carotenoid content.

Pinnipedia (sea lion, walrus, harbor seal). All animals received a mix of fish including herring, capelin, smelt and trout. Like the dolphins, they were supplemented with vitamin E and thiamin. These diets were classified as moderate in carotenoids due to the high carotenoid content of the trout.

Proboscidae (African elephant, Asian elephant). Elephants consumed primarily timothy hay (90%) and low fiber herbivore pellets (7%). Bread, apple, orange, carrot, sweet potato, banana and onion made up the remaining 3%. These diets were low to moderate in carotenoids.

Hyracoidea (rock hyrax). A high fiber mix of complete nonpurified diet (mouse and rabbit), low fiber herbivore pellets and high fiber monkey biscuit comprised 50% of a rock hyrax diet. Lettuce, kale, celery, spinach, carrot and sweet potato made up the rest. Hyrax diets had high carotenoid content.

Perissodactyla (Grant's zebra, Grevy's zebra, South American tapir, black rhinoceros). Eighty percent of the zebra diet was timothy hay; 20% was low fiber herbivore pellets. Tapir diets were 25% timothy hay, 25% low fiber herbivore pellet and 50% fruits and vegetables on an as-fed basis. Black rhinos consumed primarily timothy hay (81%). Low fiber herbivore pellets and fresh produce made up 10 and 9% of the diet, respectively, as fed. These diets were moderate in carotenoid content.

Artiodactyla (warthog, pygmy hippopotamus, Pere David's deer, Bactrian camel, okapi, giraffe, sitatunga, greater kudu, eland, Congo buffalo, banteng, wisent, addax, klipspringer, ibex). The warthog received 5% timothy hay, 65% low fiber herbivore pellets plus 30% carrot, apple and sweet potato. The diet was moderate in carotenoid content. Other hoofed animals received 80–90% alfalfa or timothy hay and 10–20% low fiber herbivore pellets. Apple, carrot, bread, banana, celery, spinach and lettuce were possible treats. These diets were low in carotenoid content.

Avian diets. *Sphenisicifomes* (Humboldt penguins). Brookfield Zoo penguins received a mix of Columbia River smelt, capelin, silver side smelt, trout and herring. They were supplemented with vitamin E and thiamin as well as a multivitamin/mineral supplement. The diet was moderate in carotenoids due to the high carotenoid content of the trout.

Pelecaniformes (brown pelican). Pelicans received a mix of fish including capelin, silver side smelt and trout and were supplemented with vitamin E, thiamin and a multivitamin/mineral tablet. The diet was moderate in carotenoids due to the high carotenoid content of the trout.

Ciconiiformes (American and greater flamingoes, scarlet ibis, hadada ibis, sacred ibis). Flamingos received a mix of 50% dog nonpurified diet, 30% duck grower (a grain-based diet that meets the nutrient needs of ducks), and 20% trout nonpurified diet (grain-based complete diet for trout), with water added to moisten. Approximately 3.3 g of a 10% canthaxanthin powder (Roxanthin Red, Hoffman LaRoche, Nutley, NJ), 0.2% of the total diet, was added to aid plumage coloration. Flamingo diets were high in carotenoid content.

Ibis received a mix of fish including capelin, silver side smelt, herring and trout. They had access to the flamingo pan as well. The ibis were supplemented with canthaxanthin only during moult. When unsupplemented, these diets are moderate in carotenoid content.

Anseriformes (Mandarin duck, wood duck, hybrid teal, whistling duck, Brazilian teal). These ducks received a 1:1:1 mix of dog nonpurified diet, duck grower and waterfowl maintenance diet (formulated to meet the needs of adult waterfowl). They also had access to fish daily and the flamingo pans from October through May, but rarely consumed either. These diets were low in carotenoid content.

Charadriformes (Gray gulls, Inca terns). Both species received a mix of fish types including Columbia River smelt, capelin, silver side smelt, trout, anchovies and herring. They were supplemented with vitamin E and thiamin as well as a multivitamin/mineral supplement. The diet was moderate in carotenoids due to the high carotenoid content of the trout.

Analytical methods. Duplicate aliquots of thawed serum were extracted twice with 2 mL hexane after ethanol deproteinization, then analyzed by HPLC using the method described by Stacewicz-Sapuntzakis et al. (1987). The carotenoids α- and β-carotene, lutein + zeaxanthin, lycopene, β-cryptoxanthin and canthaxanthin were measured. *Cis/trans* isomers of these carotenoids were not separated.

Animal foods commonly used at Brookfield Zoo (hay, horse liver, canine diet, feline diet and various fish) also were chemically analyzed to supplement published values. Food samples were blended with equal volumes of distilled water and methanol, containing 2% pyrogallol. Aqueous KOH solution (10 mol/L) was then added to produce a final concentration of 0.83–1.67 mol/L KOH; the mixture was saponified for 1 h at 70° C. The samples were then exhaustively extracted with hexane (Schriner et al. 1992). Serum and food extracts were analyzed using an HPLC system that included a 150 mm × 3.9 mm Nova-pak C-18 (5 μm) column with a Guard-pak precolumn filter from Waters (Milford, MA). α-Carotene, β-carotene and lycopene standards were obtained from Sigma Chemical (St. Louis, MO); lutein, zeaxanthin and β-cryptoxanthin were from Extrasynthese (Genay, France). The detection limits for the assay were 5 nmol/L for lutein, 9 nmol/L for β-cryptoxanthin, 9 nmol/L for lycopene and α-carotene, and 19 nmol/L for β-carotene using 200 μL of serum and a 10-μL injection. On the basis of 14 runs of control serum, coefficients of variation ranged from 1.2% for α-carotene to 7.4% for lycopene. The assay measured lutein + zeaxanthin in one peak and is referred to as lutein in this text because lutein predominates. The laboratory is a reference laboratory for the National Institute of Standards and Technology (Gaithersburg, MD) quality assurance program for carotenoids.

Dietary analysis. Quantification of dietary carotenoids was based on the "typical" diet offered to each group of animals. Many animals were housed in group situations, making it difficult to determine individual daily consumption. The assumption was made that, over time, each animal would consume its portion of the total diet offered, but individual intake was not quantified. Dietary carotenoid intakes, where available, were based on calculated concentrations per kilogram of diet offered for one animal. Dietary β-carotene was calculated using the Animal Nutritionist software program (N-squared Computing, Durango, CO) and chemical analysis of the following commonly used dietary items: alfalfa hay (4.9 mg β-carotene/kg and 35.2 mg lutein/kg), timothy hay (5.3 mg β-carotene and 47.4 mg lutein/kg), meat diets (canine, 0.6 mg β-carotene/kg; feline, 0.5 mg β-carotene/kg) and horse liver (15.9 mg β-carotene/kg).

These data were used to classify the diets on a dry matter basis as low (0.0–10 mg/kg diet), moderate (11–60 mg/kg diet) or high (61–159 mg/kg diet with one diet at 600 mg/kg) in carotenoids. These divisions were determined by examining total dietary carotenoid intakes for each species for readily apparent break points.

Data analysis and statistics. Where possible, means for species were calculated from individual serum concentrations. Multiple samples for a single individual were averaged to one value, then averaged with the other individuals for that species. Ranges were also reported when there was more than one individual.

Assessment of accumulation was based on matching diet intake to the sum of serum carotenoid concentrations for each animal species. High (++) and moderate (+) accumulators were defined as animals that had high or moderate serum carotenoid concentrations (>225 nmol/L), when provided with carotenoids in their diet. Nonaccumulators (0) were defined as animals that had negligible serum carotenoid concentrations when their dietary intake was moderate to high. If a species had low or no detectable concentrations of serum carotenoids and negligible amounts of carotenoids in their diet, they were classified as unknown (unk).

Comparisons by gender and age (less than vs. ≥7 y) for the baboon population were performed using the ANOVA statistics function (Excel, version 6.0, Microsoft, Redmond, WA) and differences were considered statistically significant at $P < 0.05$.

RESULTS

Individual serum carotenoids. **Table 1** presents mean serum concentrations of individual carotenoids for all animals collected as well as representative human values for comparison. Although inferential statistical analysis was not possible, patterns by order and species were readily apparent.

Primates. Primates had the widest distribution of carotenoids, but varied greatly among species even when consuming a similar diet. Differences in serum carotenoid concentrations could not be explained by classifying species as Old World primates vs. New World primates or apes vs. monkeys. Several species of New World monkeys possessed low to undetectable concentrations of serum carotenoids, including the golden lion tamarin and callimico, as well as the capuchin monkey. However, the spider monkey had moderate to high concentrations. The sooty mangabey, an Old World primate, had the highest serum concentration of nearly every carotenoid measured. Lutein + zeaxanthin concentrations were extremely high, compared with other mammals, at 1791 nmol/L. β-Carotene concentrations also were high (3237 nmol/L). The orangutan was within ranges noted for humans (Stacewicz-Sapuntzakis et al. 1987) in all carotenoids measured except α-carotene for which it was above the human range. Serum concentrations of all carotenoids were much lower in the gorillas than in the orangutans even though diet intakes were similar.

Among the primate species, baboons were best represented, with the largest number of individuals examined. Baboon serum lutein + zeaxanthin and α-carotene concentrations were close to those of humans and showed little variation among individuals for any carotenoid except lutein. Gender had little effect on serum carotenoid concentrations in baboons, except that males had higher levels of α-carotene than females (63.3 ± 15.3 vs. 44.1 ± 16.4 nmol/L, respectively). There was little gender difference when total serum carotenoid concentrations were examined. Males had 447 nmol/L total serum carotenoids (range 351–863 nmol/L), whereas females averaged 469 nmol/L (range 294–794 nmol/L). The younger (<7 y old) baboons had a greater serum response to diet and thus higher concentrations of serum carotenoids than those ≥7 y of age (511 ± 170 and 374 ± 79 nmol/L total carotenoids, respectively, $P = 0.022$). Total carotenoids ranged from a low of 295 nmol/L in a 19-y-old female to a high of 863 nmol/L in a 2.5-y-old male.

Marine mammals. Free-ranging dolphins had low, highly variable concentrations of serum lutein + zeaxanthin (10.6 nmol/L) and β-carotene (33.5 nmol/L), whereas captive dolphins had no detectable concentrations of any carotenoid. Like the captive cetaceans, the sea lions, harbor seals and walrus had no detectable levels of serum carotenoids.

Felids. Surprisingly, β-carotene was found in high concentrations in feline serum, but it was the only carotenoid detected. Of the 38 individual cats sampled, only two (a pallas cat and a caracal) had undetectable concentrations of β-carotene. Mean β-carotene concentrations ranged from 100 nmol/L in the fishing cats to 1058 nmol/L in the jaguars. The lowest concentration in big cats (clouded leopards, snow leopards, jaguars, tigers, lions) was 479 nmol/L β-carotene in the lions, higher than the U.S. average human serum β-carotene concentration. Small cat (all other cats in the survey) data fell into two groups, those with β-carotene concentrations <233 nmol/L (pallas cats, bobcats, caracals, fishing cats, margays) and those with concentrations >540 nmol/L (sand cat, ocelot, Geoffroy's cat). The higher concentrations in the small cats were from single individuals; thus it is difficult to determine if this is characteristic of these species. Coefficients of variation within species were greatest for the pallas cats and smallest for the snow leopards.

Non-felid carnivores. Non-felid carnivores, including wolves, cape hunting dogs and bears, had no detectable concentrations of any carotenoids when consuming low to moderate amounts of β-carotene in their diet. A single sample from a sloth bear had a lutein concentration of 12.31 nmol/L, whereas the other sloth bear had none.

JN THE JOURNAL OF NUTRITION

Ungulates (Perissodactyla and Artiodactyla). With some exceptions, the wide range of taxa which were grazers and browsers and thus consuming moderate amounts of lutein and β-carotene even in captivity, had nondetectable serum concentrations of any carotenoid. Grant's zebra and the South American tapir had low concentrations of lutein, α-carotene (tapir only) and β-carotene. Seven samples from three black rhinoceros had no detectable serum carotenoid concentrations despite moderate intakes of lutein and β-carotene.

Ungulates had mainly undetectable to negligible serum carotenoid concentrations despite moderate intakes of carotenoids. Only the banteng (2073 nmol/L β-carotene) and wisent (2250 nmol/L β-carotene), both bovids, had high concentrations of carotenoids but low concentrations of lutein despite much higher dietary lutein + zeaxanthin. In the warthog, α- and β-carotene were detected in the serum, with β-carotene concentrations of 171 nmol/L, despite the warthog's membership in the suidae family, which are reputedly white fat animals.

Birds. Within the birds, *Sphenisciformes, Pelecaniformes* and *Charadriformes* (penguins, pelicans, gulls and terns) had mostly lutein + zeaxanthin in their serum. Gulls (2478 nmol/L) and terns (1252 nmol/L) had the highest concentrations of lutein + zeaxanthin. Gull serum also contained cryptoxanthin, but no other carotenoids were detected. Ducks showed selectivity toward the more water-soluble carotenoids, lutein + zeaxanthin, canthaxanthin and cryptoxanthin. Mandarin ducks had the highest concentrations of lutein (3230 nmol/L) noted for any species. Canthaxanthin (a dietary supplement) was found at very high concentrations in the serum of flamingos, whereas lutein + zeaxanthin was not detectable, possibly because of the extremely large peak of canthaxanthin, which impaired HPLC separation of oxycarotenoids. Ibis had high concentrations of both lutein + zeaxanthin and canthaxanthin in their serum.

Classification by accumulation: total serum carotenoids and dietary intake. Taxa within each order were ranked according to the concentration of total carotenoids found in their serum. The means and ranges for each species are listed in **Table 2** as well as the dietary carotenoid classification and accumulation rating.

In general, birds accumulated high concentrations of dietary carotenoids regardless of the amount in the diet. These were exclusively xanthophylls even though most bird diets contained β-carotene. Exceptions include the sacred ibis, Humboldt penguin and Brazilian teal, which were designated as moderate to low in carotenoid accumulation.

Primates varied in dietary carotenoid accumulation from high accumulators such as the sooty mangabey and orangutan to the callimico, capuchin and golden lion tamarin, which had low accumulation despite moderate intakes of dietary carotenoids. It is interesting to note that although the spider monkey consumed a diet nearly identical to that of the capuchin, it had high accumulation. What distinguished the primates as a group from other orders was their accumulation of both carotenes and xanthophylls.

Ungulates do not appear to accumulate xanthophylls; their accumulation of carotenes is indeterminate because they had undetectable serum concentrations of either carotenoid class and moderate dietary lutein + zeaxanthin but low dietary β-carotene. The exceptions were the wisent and banteng, which had high serum carotenoid concentrations despite moderate dietary intakes. Warthogs had moderate carotenoid accumulation; both α- and β-carotene were detected in their serum. Warthogs consumed a diet similar to other hoofstock, but higher in fruits and vegetables.

Felines were all classified as high accumulators. Pallas cats, caracals, bobcats and fishing cats had moderate serum concentrations despite low dietary carotenoids.

Most other carnivores and marine mammals were classified as unknown or low in their accumulation because of low or moderate dietary carotenoids and undetectable or low serum concentrations.

DISCUSSION

The remarkable variation in serum carotenoid types and concentrations among species cannot be explained completely by differences in dietary intake or in taxa. In primates, which appear to accumulate the largest array of carotenoids, variation included the type of carotenoid found in the serum as well as its concentration. All primate diets were relatively high in the measured carotenoids except lycopene, which was a negligible dietary component. In general, primates had higher serum lutein concentrations than humans, but lower concentrations of other carotenoids. Exceptions were the sooty mangabey and the orangutan, which had higher concentrations of all carotenoids except lycopene than an average U.S. human consuming a diet rich in fruit and vegetables (Henderson et al. 1989). The gorillas had lower concentrations than the orangutans. Because of their close taxonomic relation, one would expect a similar accumulation of dietary carotenoids. Although diets fed to gorillas and orangutans in captivity were very similar, in the wild, these animals evolved different feeding strategies. The free-ranging gorilla eats primarily leafy matter, whereas orangutans are mainly frugivorous. It is possible that different feeding strategies led to differences in the ability of these animals to accumulate carotenoids.

In general, a baboon consuming a diet similar to that of the sooty mangabey had much lower carotenoid concentrations, and individual differences also were apparent. For example, five sooty mangabeys ranged from 1773-4392 nmol/L in serum β-carotene, while showing little difference in β-cryptoxanthin. Similarly, Snodderly et al. (1990) compared plasma carotenoids of the macaque and squirrel monkey fed the same diet. Macaque plasma concentrations were nearly twenty times greater for β-cryptoxanthin than squirrel monkey plasma.

There have been questions regarding the relationship between the color of mammalian fur (especially the red/orange-haired primates) and their level of carotenoid accumulation. We have assessed carotenoid concentrations of orangutan and golden lion tamarin hair, the two species with especially orange hair, and have found them to contain no detectable concentrations of carotenoids.

Our baboon males had higher concentrations of α-carotene, but little gender difference was seen in total carotenoid concentrations. Gender differences have been reported in humans, with middle-aged women having higher serum concentrations of β-cryptoxanthin and β-carotene than middle-aged men (Stacewicz-Sapuntzakis et al. 1987). These differences in humans have been attributed to greater fruit and vegetable consumption among women (Ascherio et al. 1992). It appeared that the younger ($<$7 y old) baboons had an increased response to diet and thus higher concentrations of serum carotenoids than those $\geq$7 y of age, although they might also have consumed more food and also dietary carotenoids. These data were in agreement with Sugerman et al. (1991) who found that young men absorbed β-carotene more rapidly, but contrast with data of Maiani et al. (1989), who found that older women had enhanced β-carotene absorption compared with younger women.

Felids may be selective accumulators of β-carotene; because other carotenoids were not in the diet, however, this would be a premature classification. "Big cat" diets were high in β-carotene because of the high proportion of liver (contrary to supplier specifications) in the prepared meat diet consumed by these cats. Smaller cats were consuming a different prepared meat, which was calculated to be low in β-carotene. Regardless of the diet consumed, all species of cat sampled had moderate to high β-carotene serum concentrations. The presence of β-carotene in feline blood is contradictory to older published reports that concluded that domestic cats do not accumulate dietary β-carotene (Ahmad 1931, Gershoff et al. 1957). Only recently has there been mention of the domestic cat possessing β-carotene in its blood (Baker et al. 1986, Chew et al. 1997). Studies by Chew et al. (1997) showed that the domestic cat absorbed significant amounts of dietary β-carotene. Studies by Lakshmanan et al. (1972) suggested that domestic cats lack the enzyme necessary to convert β-carotene to vitamin A. The ability of exotic felids to convert β-carotene to vitamin A has not been studied. The inability of the domestic cat to convert β-carotene to retinol raises the question of whether absorbed β-carotene can be utilized by exotic felids. High circulating concentrations may be a by-product of negligible conversion to retinol, but this remains unknown. It is also possible that more sensitive methods of retinol analysis will allow for detection of some conversion at concentrations that were previously undetectable.

Ungulates had primarily undetectable to negligible serum carotenoid concentrations despite their consumption of diets moderate in carotenoid content except the banteng and wisent. The wisent had the highest plasma β-carotene concentration (2250 nmol/L) of any mammal sampled. All hoofed mammals were offered a combination of hay and a pelleted herbivore diet that contained 20 mg β-carotene/kg. Hay provided β-carotene (4.8–5.3 mg/kg) and high concentrations of lutein + zeaxanthin (35.2–47.4 mg/kg). Studies by Anderson et al. (1991) found that domestic steers had β-carotene concentrations of 5547–8645 nmol/L. Domestic bovid β-carotene serum concentrations are more than double that of the banteng and wisent. The domestic bovids sampled may have had considerable fresh grass in their diets. These data agree with the suggestion that bovids are selective carotene accumulators (Goodwin 1984). It is interesting to note that the Congo buffalo, also a bovid, had no detectable serum concentrations of any carotenoid despite a similar diet. An evaluation of free-range vs. captive ungulate diets and carotenoid plasma concentrations would be interesting because fresh grass and tree leaves are only negligibly available to captive ungulates in Northern zoos.

In the warthog, both α- and β-carotene were detected in the serum. This is contrary to studies performed by Poor et al. (1987) that showed that the domestic pig had no detectable concentrations of serum β-carotene despite a diet supplemented with β-carotene instead of retinol (5.2 mg β-carotene/kg).

Many of these exotic hoofstock sampled (mainly *Artiodachyla*) were not of the *Bovinae* subfamily, and may be more closely related taxonomically to sheep or goats than to cows. Goats do not have carotenoids in their blood or tissues (Goodwin and Gregory 1948) and sheep usually have only low levels (Pierce 1946). Goodwin and Gregory (1948) suggested that carotene was converted to vitamin A in sheep, but not absorbed intact. This could explain why there were no detectable concentrations of carotenoids in these animals. Schweigert (1995) suggested that further research be conducted concerning carotenoid supplementation of herbivorous species such as ungulates.

The horse-related species (*Perissodactyla*) had carotenoids ranging from not detectable in the black rhinoceros to 233 nmol/L in the Grant's zebra. Lutein was found in both the Grant's zebra and tapir serum, but only the tapir had α-carotene. The diets offered were classified as moderate in carotenoids, which makes these animals moderate or, in the case of the black rhino, low accumulators. The serum concentration of β-carotene in the Grant's zebra was similar to the 261 nmol/L reported in horses by Baker et al. (1986), but considerably lower than the 939 nmol/L carotene reported for horses by Vander Noot et al. (1964). Horse-related species that accumulate may be selective carotene accumulators like the bovids.

Canids (dogs and wolves), Mustelids (otters), Ursids (bears) and Viverrids (binturong) were classified as low accumulators. They had no detectable concentrations of serum carotenoids while consuming diets that were low to moderate in carotenoid content. Studies conducted with domestic dogs have shown low to moderate concentrations of circulating β-carotene (Baker et al. 1986, Frohring 1935, Steenbock et al. 1921, Turner 1934). Recent studies by Weng et al. (1997) showed absorption of significant amounts of dietary β-carotene and uptake by lymphocytes and neutrophils in domestic dogs.

Captive dolphin serum contained no measurable carotenoids. Cetacean carotenoid concentrations have not been quantified in the literature. Like the captive cetaceans, the sea lions, harbor seals and walrus had no detectable concentrations of the serum carotenoids measured. We did not measure astaxanthin, a very polar carotenoid accumulating in crustacea and some fish such as salmon. Our data were consistent with that of Schweigert et al. (1987) who found no detectable concentrations of β-carotene among 65 free-ranging Grey seals. Brookfield Zoo's marine mammal diets are moderate in carotenoid content, classifying these animals as nonaccumulators. Because free-ranging dolphins had low concentrations of lutein and β-carotene in their serum, this should be investigated further. The presence of lutein + zeaxanthin (from green, vegetative sources) in free-ranging dolphins raises the issue of whether dolphins may feed directly on plant material as well as fish or if the source of lutein + zeaxanthin is from the live fish consumed. Perhaps the handling and freezing process for commercial fish causes the fish gastrointestinal tract to empty thus reducing the lutein content of frozen fish, or perhaps the fish fed to captive animals do not consume plankton, which are high in carotenoids.

Our data were consistent with the understanding that all birds appear to be selective accumulators of oxycarotenoids. Much research has been conducted in the poultry industry regarding xanthophylls and their use in improving coloration of egg yolks and chicken tissues, as reviewed in Marusich and Bauernfeind (1981). Carotenoid supplementation of captive wild birds to improve plumage coloration has been practiced for many years. Lutein + zeaxanthin concentrations were high in the majority of avian serum samples, despite only moderate intakes of lutein and β-carotene. When it was available, birds also accumulated canthaxanthin. Most flamingo diets are supplemented with carotenoids to improve plumage coloration, including those at Brookfield Zoo. The mean carotenoid concentrations that we reported (35 μmol/L) were similar to those of 23–56 μmol/L reported by Fox and co-workers (1966 and 1969). The mandarin duck, although receiving a low carotenoid diet, had high serum concentrations of lutein + zeaxanthin. There are no available data regarding carotenoid accumulation in ducks.

Health status. It is unknown how the health of an animal affects serum carotenoid concentrations. The majority of samples (~95%) for this study came from animals that were examined as part of routine or preshipment physicals and thus were healthy. However, many of the samples from hoofstock were from old animals being killed because of complications of age and illness.

There is some case evidence that illness may affect serum carotenoid concentrations. A spectacled langur had extreme malabsorption problems and was treated for a possible phosphorus deficiency. She had no detectable concentrations of any carotenoid except lutein, and it was extremely low compared with the rest of the langur group (11.43 vs. 165.3–1282 nmol/L).

Only three samples were taken from sick felids. Two of the three Siberian tigers were ill and eating poorly. Their β-carotene concentrations were approximately half that of the healthy animal. An aged and ill lion exhibited β-carotene concentrations that were one third that of his healthy values, but not noticeably different from that of two young male lions. Health status may affect carotenoid concentrations, but it is difficult to separate poor intake and treatment effects from that of the illness. Total serum carotenoids have long been used as a clinical test for malabsorption in humans (Miller 1985). These observations may argue for the use of reference carotenoid concentrations for healthy animals that accumulate carotenoids as an aid in diagnosis and therapy in illness.

Metabolism and tissue disposition. The extent to which plasma or serum carotenoid composition and concentration reflects those of solid tissues is not known, but may be of importance in determining carotenoid "status" and function. Many factors may influence this relationship. Tissues are likely to reflect months or years of consumption, whereas plasma concentrations may reflect only days or weeks (Parker 1989). Nevertheless, the pattern and concentration of carotenoids in plasma reflect most tissue concentrations in humans (Parker 1989).

Yang and Tume (1993) suggested that differences in the selective absorption processes in the small intestine are responsible for the various concentrations of carotenoids observed in different species of animals. It is generally thought that carotenoids move into the enterocyte by passive diffusion (Furr and Clark 1997). If this is the case, species differences in absorption/accumulation may arise due to species differences in gut luminal events such as diversity in pH, gut motility and liposome and micelle formation, as well as the wide range in the type and amount of dietary fat. In addition, little is known concerning how carotenoids are incorporated into lipoprotein fractions and enter the circulation (Furr and Clark, 1997). Species variations in lipoprotein handling are also likely to be large contributors to differences in the accumulation of carotenoids. Small differences in all of the factors known to affect carotenoid absorption (de Pee and West 1996) could translate into the large differences in accumulation among species and individuals within species.

Conclusions and questions for future research. Given the selectivities described in this paper for specific carotenoids and unique accumulation patterns among various exotic species, it appears that animals have developed a variety of strategies for absorption, clearance and utilization of carotenoids. These may include conversion to vitamin A, accumulation in skin and plumage for communication and reproduction, antioxidant strategies and the hormone-like activity important in bovid fertility. Despite these uses by accumulators, nonaccumulators appear to fare well in the absence of intact carotenoids although their conversion to vitamin A is essential to life. These differences raise interesting issues. If the accumulation of intact carotenoids is important for animals and may confer some special advantage, why do the types of carotenoids found in blood vary so widely among species? What is the mechanism by which birds accumulate xanthophylls and not carotenes? Why are the primates unique in accumulating a wide range and high concentrations of carotenoids, whereas many other mammals accumulate only one kind of carotenoid or none? These questions and others concerning carotenoid metabolism and function are still to be explored. Captive exotic animals as well as their free-range counterparts provide a possible window for further inquiry into these questions.

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