Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 7627–7631, December 1984
Medical Sciences

Carotenoids and retinol: Their possible importance in determining longevity of primate species

(aging/antioxidants/free-radicals/cancer/anticarcinogens)

RICHARD G. CUTLER

Gerontology Research Center, National Institute on Aging, Francis Scott Key Medical Center, Baltimore, MD 21224

Communicated by J. Edwin Seegmiller, August 17, 1984

ABSTRACT **Aging and cancer share a number of characteristics. This has led to the hypothesis that species' differences in longevity may be governed in part by the same mechanisms as those processes governing species' differences in their age-dependent probability of developing cancer. Much evidence has indicated that β-carotene and retinol may be important natural anticarcinogens. Accordingly, they also may be important antiaging agents. This possibility has been tested by determining if a positive correlation exists between the concentration of carotenoids and retinol in serum and brain tissue with the maximal life-span potential of mammalian species. The results show a significant positive correlation for the carotenoids but not for retinol. These results suggest that the carotenoids may be biologically active as protective agents against cancer and as longevity determinants. Retinol appears to be less important in these functions.**

Considerable epidemiological and laboratory evidence exists suggesting that retinol and β-carotene may be natural protective agents against cancer development (1–5), but definitive experiments have not yet been reported. Retinol has a wide range of biological effects, particularly on cell growth and differentiation (6–10), and it is also an antioxidant (11, 12). Carotenoids may have a protective role for a wide variety of plants and microorganisms (13–15), but their biological role in mammals is less clear (13, 16–18). β-Carotene is an excellent singlet-oxygen scavenger and can protect against photosensitized oxidation (19–21). More recently, β-carotene has been shown to protect against free-radical-induced lipid peroxidation (22–24) and to be a good radical-trapping antioxidant at an oxygen partial pressure typically found in normal tissues (25). There is no evidence for a biological role of the xanthophylls in mammalian species (16–18). Thus, in mammalian species, carotenoids could be an important natural protective agent against the oxygen radicals that are produced in all tissues as normal by-products of metabolism (26–28).

Nothing definite is known about the biological basis determining the maximal life-span potential (MLSP) of mammalian species, but it may involve the same factors that determine the sensitivity of different species to cancer (26, 29, 30). Thus, β-carotene and retinol may be protective against both aging and cancer through a common mechanism. This possibility has been investigated by determining if a positive correlation in the concentrations of carotenoids and retinol in serum and brain tissues exist as a function of the MLSP of six primate and seven nonprimate mammalian species. A significant positive correlation was found for the carotenoids but not for retinol.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Source of Tissues. Primate blood and brain samples were obtained from Yerkes Regional Primate Center, Duke University Primate Facility, Washington Regional Primate Center, and the Delta Regional Primate Center. Serum samples for nonprimate species were obtained from Pel-Freez. Human serum samples were taken from volunteers, and blood and tissue samples from *Peromyscus maniculatus* (deer mouse) and *Mus musculus* (field mouse) were from our wild-type animal colony at the Gerontology Research Center.

Estimation of Species' Longevity. Estimates of MLSP were taken from a literature survey covering over 100 zoos worldwide (31, 32).

Life-Span Energy Potential (LEP). LEP is calculated as the product of MLSP and basal specific metabolic rate (SMR) and represents the energy expenditure over MLSP on a per unit weight basis (26–28, 33).

Carotenoid and Retinol Assays. Blood samples were collected in the morning after an overnight fast and were protected from excessive light exposure. The serums were immediately prepared and frozen at −70°C. Total carotenoids (carotene and xanthophylls) and retinol were measured in the serum by using the assay of Kahan (34, 35).

Carotenoids and retinol were measured in brain samples by using the method described by McLaren *et al.* (36). Lipids were extracted with chloroform/methanol, and the solution was dried by evaporation (37).

RESULTS

Carotenoid and Retinol Concentration in Serum. Serum concentrations of carotenoids and retinol are tabulated in Table 1 and illustrated in Figs. 1 and 2. Fig. 1*a* shows a positive correlation of serum carotenoid concentration as a function of species MLSP, where $r = 0.835$ ($P < 0.001$) for all 12 species and, for the primates, $r = 0.936$ ($P < 0.01$).

In contrast, Fig. 1*b* shows serum retinol concentration to be remarkably constant for the different mammalian species, where the linear correlation coefficient for all species is $r = 0.363$ ($P > 0.1$) and for the primates is $r = 0.163$ ($P > 0.1$). Thus, the ratio of carotenoids per retinols, as shown in Fig. 1*c*, is seen to increase significantly as a function of MLSP, where the linear correlation coefficient for all species is $r = 0.612$ ($P < 0.05$) and for primates is $r = 0.905$ ($P < 0.02$).

Fig. 2 shows the results if the data were plotted against LEP instead of MLSP. The results are remarkably similar, but the correlation coefficients for the primates are slightly less significant, as indicated in the legend.

Age-Dependent Changes in Carotenoids and Retinol. Serum carotenoid or retinol concentrations as a function of age are

Abbreviations: MLSP, maximal life-span potential; LEP, life-span energy potential.

Table 1. Serum concentrations of carotenoids and retinol in primate and nonprimate mammalian species as a function of MLSP and LEP

Ident. no.	Species (common name)	MLSP, yr	LEP, kcal/g	Carotenoids, μg/100 ml	Retinol, μg/100 ml	Ratio carotenoids/ retinol
			Primates			
1	Human	90 ± 5	815 ± 51	54.8 ± 10.5 (n = 10)	24.0 ± 3.68 (n = 10)	2.03 ± 0.498
2	Orangutan	50 ± 8	447 ± 79	34.8 ± 13.5 (n = 4)	30.2 ± 4.25 (n = 4)	1.15 ± 0.473
3	Chimpanzee	48 ± 5	469 ± 51	17.8 ± 11.3 (n = 15)	24.6 ± 4.78 (n = 16)	0.726 ± 0.481
4	Gorilla	43 ± 5	309 ± 36	10.6 ± 2.42 (n = 3)	18.7 ± 5.71 (n = 3)	0.566 ± 0.215
5	Gibbon	35 ± 8	569 ± 42	4.4 ± 2.1 (n = 3)	31.3 ± 3.02 (n = 3)	0.140 ± 0.0680
6	Rhesus	34 ± 5	512 ± 76	9.9 ± 3.26 (n = 4)	11.6 ± 1.25 (n = 4)	0.853 ± 0.295
			Nonprimate mammals			
7	Horse	46 ± 8	235 ± 20	21.4 ± 3.07 (b = 3)	9.39 ± 4.18 (b = 3)	2.27 ± 1.06
8	Cow	30 ± 5	164 ± 15	36.0 ± 2.03 (b = 2)	14.5 ± 0.494 (b = 2)	2.48 ± 0.163
9	Goat	25 ± 5	277 ± 30	3.3 ± 1.27 (b = 2)	18.2 ± 1.31 (b = 2)	0.181 ± 0.0708
10	Rabbit	12 ± 2	257 ± 30	2.40 ± 1.69 (n = 2)	20.6 ± 0.919 (n = 2)	0.116 ± 0.0817
11	Deer mouse	8 ± 1	440 ± 68	1.32 ± 1.76 (n = 5)	16.9 ± 7.48 (n = 5)	0.078 ± 0.109
12	Rat	4.0 ± 0.5	152 ± 30	1.65 ± 2.91 (b = 4)	16.2 ± 4.54 (b = 4)	0.101 ± 0.315
13	Field mouse	3.5 ± 0.5	232 ± 15	1.65 ± 1.13 (n = 4)	14.9 ± 4.21 (n = 4)	0.110 ± 0.0814

Ident. no., identification number; n, no. of different individuals measured; b, number of separate serum batches analyzed. (Each serum batch represents pooled serum samples from an unknown number of different individuals of unknown ages.)

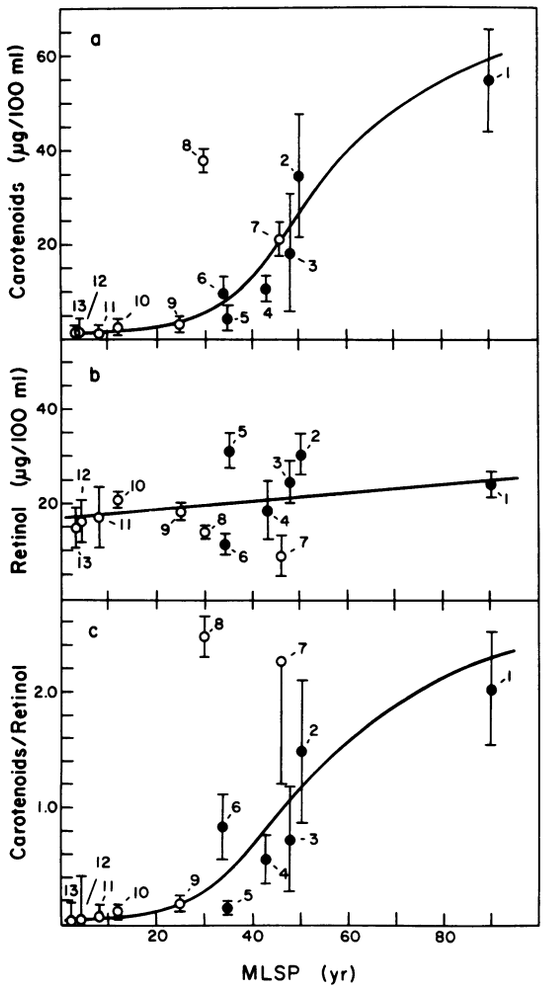

FIG. 1. Serum carotenoid and retinol concentrations as a function of MLSP. ●, Primates; ○, nonprimate species. Identification numbers of species are listed in Table 1. Error bars represent SD. For primates only, linear correlation coefficients are: $r = 0.926$ ($P < 0.010$) in a, $r = 0.163$ ($P > 0.10$) in b, and $r = 0.905$ ($P < 0.020$) in c.

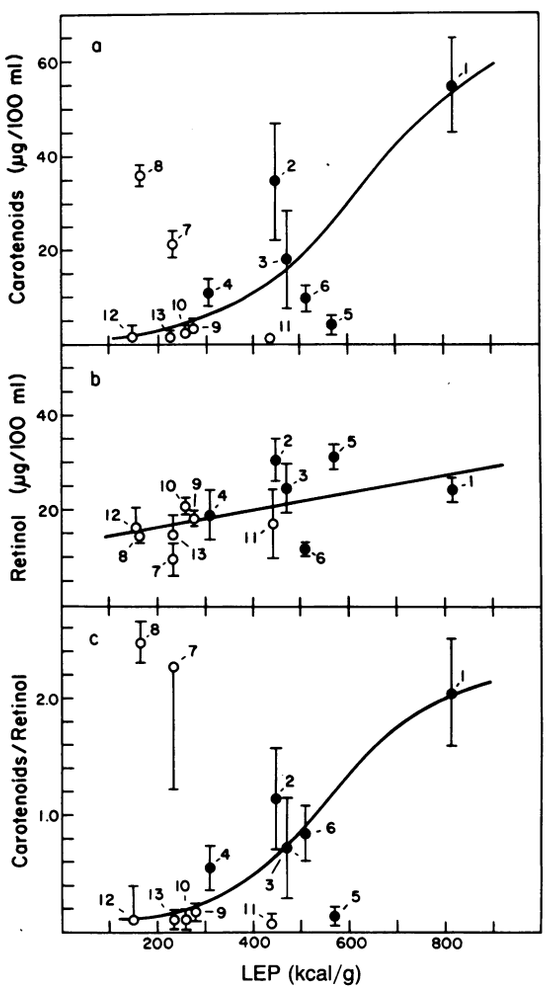

FIG. 2. Serum carotenoid and retinol concentrations as a function of LEP. ●, Primates; ○, nonprimate species. Identification numbers of species are listed in Table 1. Error bars represent SD. For primates only, linear correlation coefficients are: $r = 0.635$ ($P > 0.1$) in a, $r = 0.175$ ($P > 0.1$) in b, and $r = 0.546$ ($P > 0.1$) in c.

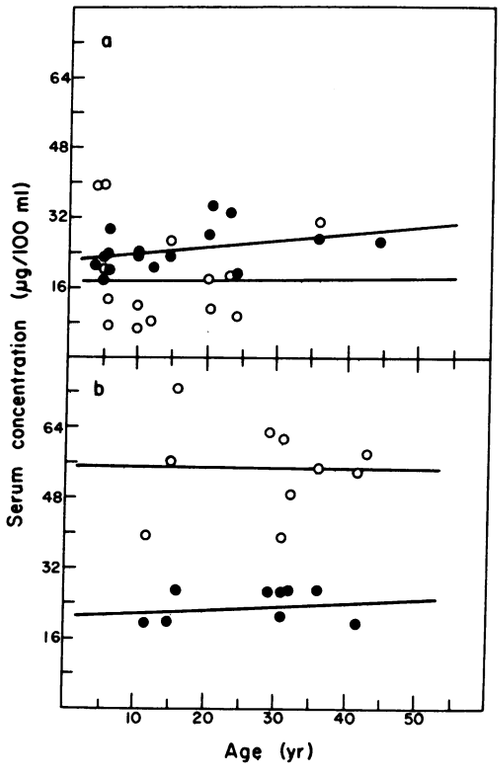

FIG. 3. Serum carotenoid and retinol concentrations in human and chimpanzee as a function of age. ●, Retinol; ○, carotenoids. (*a*) Chimpanzee; carotenoids, $r = 0.023$ ($P > 0.1$); retinol, $r = 0.39$ ($P > 0.1$). (*b*) Human; carotenoids, $r = -0.028$ ($P > 0.1$); retinol, $r = 0.25$ ($P > 0.1$).

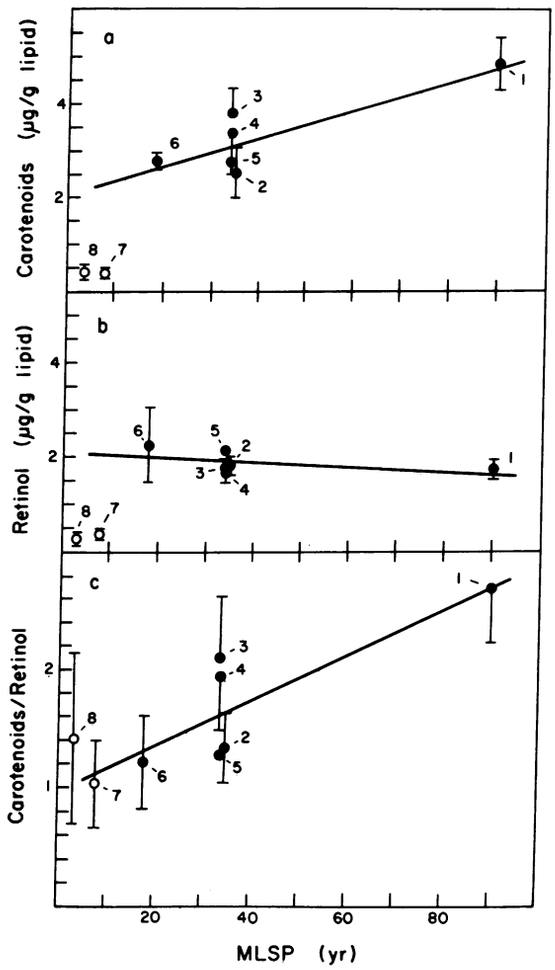

FIG. 4. Brain carotenoid and retinol concentrations as a function of MLSP. ●, Primate species; ○, rodent species. Identification numbers for species are listed in Tables 2 and 3. For primates only, linear correlation coefficients are $r = 0.859$ ($P < 0.050$) in *a*, $r = -0.432$ ($P > 0.1$) in *b*, and $r = 0.822$ ($P < 0.050$) in *c*.

shown in Fig. 3 for human and chimpanzee and indicate that no significant changes occur in either carotenoid or retinol serum concentration as a function of age. These results confirm other studies of human carotene and retinol levels as a function of age (38).

Carotenoid and Retinol Levels in Brain. Results are tabulated in Table 2 and illustrated in Figs. 4 and 5. The concentrations of the carotenoids and retinol are presented both on a wet-weight and on a lipid-weight basis. A significant positive correlation of brain carotenoid concentration was found as a function of either MLSP and LEP values. In contrast to the serum data, the correlations of carotenoid concentration in the brain with the species' LEP values appear to be slightly better than with MLSP. However, as with the serum, retinol concentration in the brain changes little as a function of MLSP or LEP, and thus again there is a significant increase in the ratio of carotenoids to retinol with MLSP or LEP value. The brain carotenoid and retinol concentrations for the two rodent species were found to be similar to one another

Table 2. Brain concentrations of carotenoids and retinol in primates

Ident. no.	Species	MLSP, yr	LEP, kcal/g	Carotenoids,* μg/100 g	Retinol,* μg/100 g	Carotenoids,† μg/g	Retinol,† μg/g	Ratio carotenoids/ retinol
1	Human	90	815	97.1 ± 12.8 $n = 4$	35.9 ± 3.55 $n = 4$	4.85 ± 0.64 $n = 4$	1.79 ± 0.177 $n = 4$	2.70 ± 0.420
2	Baboon	35	394	42.5 ± 8.5 $n = 6$	31.4 ± 3.4 $n = 6$	2.50 ± 0.501 $n = 6$	1.85 ± 0.200 $n = 6$	1.35 ± 0.307
3	Rhesus	34	512	76.8 ± 11.8 $n = 4$	36.3 ± 7.68 $n = 4$	3.74 ± 0.575 $n = 4$	1.77 ± 0.374 $n = 4$	2.11 ± 0.550
4	Stump-tailed macaque	34	512	85.0 ± 23.1 $n = 4$	43.4 ± 4.3 $n = 4$	3.29 ± 0.896 $n = 4$	1.68 ± 0.166 $n = 4$	1.95 ± 0.564
5	Crab-eating macaque	34	512	42.6 $n = 1$	33.4 $n = 1$	2.73 $n = 1$	2.14 $n = 1$	1.27
6	Squirrel monkey	18	485	72.3 ± 2.96 $n = 2$	58.9 ± 19.3 $n = 2$	2.76 ± 0.113 $n = 2$	2.24 ± 0.737 $n = 2$	1.22 ± 0.404

Ident. no., identification number.
*Per 100 g of wet weight tissue.
†Amount found in whole tissue normalized by the weight of total lipids.

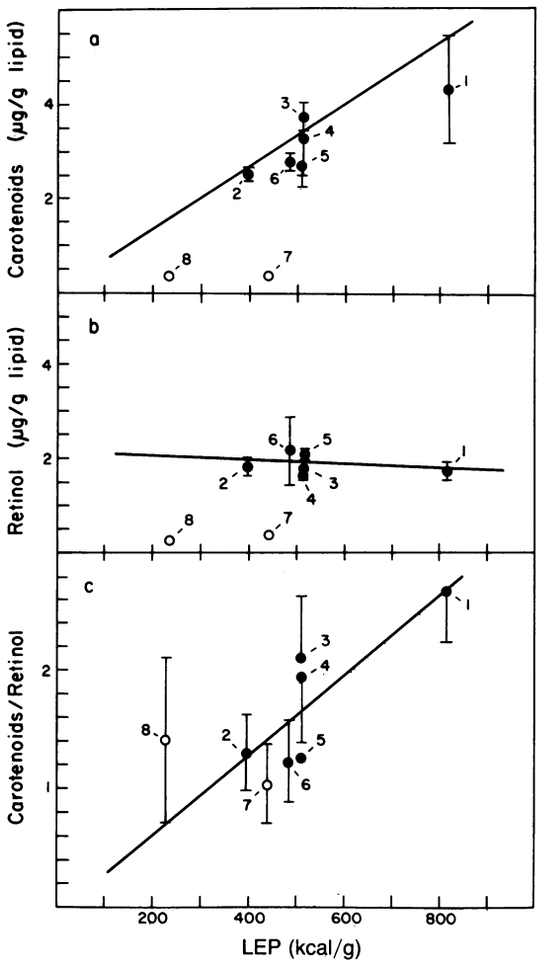

FIG. 5. Brain carotenoid and retinol concentrations as a function of LEP. ●, Primate species; ○, rodent species. Identification numbers for species are listed in Tables 2 and 3. For primates only, linear correlation coefficients are $r = 0.920$ ($P < 0.010$) in *a*, $r = -0.239$ ($P > 0.1$) in *b*, and $r = 0.821$ ($P < 0.050$) in *c*.

and much lower than the longer-lived primate species.

***Peromyscus* and *Mus* Comparison.** *Peromyscus maniculatus* (deer mouse) is an unusually long-lived rodent species, having a MLSP of about 8 yr and a LEP value of about 440 kcal/g (1 cal = 4.184 J). On the other hand, *Mus musculus* (field mouse) has a relatively short life-span of 3.5 yr and a lower LEP value of about 232 kcal/g. Both animals weigh approximately 25 g and have similar basal specific metabolic rates. Since a number of different tissues were available from these animals, a comparison of their carotenoids and retinol concentrations was made. These data are shown in Table 3. The results show no significant differences between these two species in the tissue concentrations of carotenoids or retinol.

DISCUSSION

The major result of these studies is that a significant positive correlation was found between the MLSP of different mammalian species and the concentration of carotenoids in serum and brain tissue but not for retinol. These results suggest that carotenoids may be important in determining both MLSP and the age-dependent probability of cancer development in mammals.

The possibility that carotenoids may act in this capacity as antioxidants was not clarified by the LEP correlation studies. Here, the correlation with MLSP or LEP was of equal significance for the serum, although the correlation with LEP was slightly better than with MLSP for the brain. Thus, the biological activity of carotenoids may be as an antioxidant and also by other unknown mechanisms which could stabilize the differentiated state of cells.

The shorter-lived mammalian species generally had low concentrations of carotenoids in serum and brain, but it was surprising to find that the concentrations in the longer-lived deer mouse were not higher than for the field mouse. These data suggest that the role of carotenoids as determinants of mammalian longevity may have evolved only in the longer-lived species, reaching a peak expression in human.

The possible importance of retinol as a natural anticarcinogen as well as retinoid derivatives being potentially useful in therapy of cancer is well known (1–5). However, no significant correlation of retinol concentration in serum or brain tissue with the species' MLSP or LEP values was found. Most importantly, human serum and brain tissue did not have similar levels of retinol compared to the shorter-lived primates. These results indicate that, although retinol may be an anti-cancer agent, it does not appear important in determining the large differences existing in MLSP or cancer resistance within the mammalian species.

It is not understood what determines the serum and tissue concentrations of carotenoids, but it is likely to be based in part on the quantitative and qualitative aspects of absorption

Table 3. Concentration of carotenoids and retinol in tissues of the field mouse and the deer mouse

Ident. no.	Species tissue	MLSP, yr	LEP, kcal/g	Carotenoids,* μg/100 g	Retinol,* μg/100 g	Carotenoids,† μg/g	Retinol,† μg/g	Ratio carotenoids/ retinol
7	Deer mouse‡	8	440					
	Brain			44.7 ± 14.1	42.5 ± 5.51	0.380 ± 0.120	0.362 ± 0.0469	1.05 ± 0.358
	Kidney			87.1 ± 25.6	117 ± 10.6	1.61 ± 0.473	2.16 ± 0.196	0.745 ± 0.229
	Heart			38.9	56.2	0.828	1.19	0.695
	Spleen			12.8	34.1	0.149	0.398	0.374
8	Field mouse§	3.5	232					
	Brain			66.6 ± 33.3	46.8 ± 3.32	0.394 ± 0.197	0.277 ± 0.0189	1.43 ± 0.721
	Kidney			157 ± 41.2	133 ± 7.07	5.29 ± 1.41	4.57 ± 0.243	1.15 ± 0.852
	Heart			41.3	70.3	0.995	1.69	0.588
	Spleen			40.0	98.5	0.300	0.738	0.406

Data represent mean ± SD of four individual animals. All animals were 6-mo males. Both species were fed identical diets ad lib. Heart and spleen tissues were pooled specimens.

*Grams represent wet tissue weight.

†Grams represent total lipid extracted by cyclohexane, dried by evaporation, and then weighed.

‡*Peromyscus maniculatus* (outbred).

§*Mus musculus* (outbred).

and on the activity of an enzyme found predominantly in the intestinal mucosa cells, β-carotene 15,15'-dioxygenase (39). This enzyme is responsible for initiating the conversion of carotene to retinol, and low levels would be expected in the longer-lived species. Humans unselectively absorb both carotenes and xanthophylls into their tissues, whereas the shorter-lived species absorb only the carotenes (16–18). The amount of carotenoids in the diet would of course also play an important role in determining the amount of carotenoids that are absorbed, but not the qualitative aspects. Thus, although all carotenoids are derived from the diet, the amount and type that is absorbed in serum and other tissues is clearly a species-dependent characteristic.

During the evolution of increased MLSP in the mammalian species, and particularly in the primates (27, 40), carotenoid concentration in serum and tissues also may have increased. This may have been facilitated by an increased absorption of both the carotenoids and xanthophyll and a decrease in the activity of intestinal β-carotene 15,15'-dioxygenase. Thus, the nonselective absorption of the carotenoids in humans may represent an end point to this evolutionary strategy; most of the carotenoid protection attainable through the diet is now being utilized.

In conclusion, these correlation studies appear to justify more definitive experiments using species having different MLSPs to further study the possible role carotenoids and retinol may have in determining human longevity and resistance to cancer.

Thanks are expressed to N. Krinsky for stimulating discussions and Edith Cutler for laboratory assistance, drawing of the figures, and typing of the manuscript. Her help was made possible by support received from the Paul Glenn Foundation for Medical Research.

1. Peto, R., Doll, R., Buckley, J. D. & Sporn, M. B. (1981) *Nature (London)* **290**, 201–208.
2. Calabrese, E. J. (1980) *Nutrition and Environmental Health* (Wiley, New York), pp. 1–93.
3. Hill, D. L. & Grubbs, C. J. (1982) *Anticancer Res.* **2**, 111–124.
4. Ames, B. N. (1983) *Science* **221**, 1256–1264.
5. Hicks, R. M. (1983) *Proc. Nutr. Soc.* **42**, 83–93.
6. Peto, R. (1983) *Proc. Nutr. Soc.* **42**, 81.
7. Kolata, G. (1984) *Science* **223**, 1161.
8. Lotan, R. (1980) *Biochim. Biophys. Acta* **605**, 33–91.
9. Newberne, P. M. & Rogers, A. E. (1981) in *Nutrition and Cancer: Etiology and Treatment*, eds. Newell, G. R. & Ellison, N. M. (Raven, New York), pp. 217–232.
10. Zile, M. H. & Cullum, M. E. (1983) *Proc. Soc. Exp. Biol. Med.* **172**, 139–152.
11. Kartha, V. N. R. & Krishnamurthy, S. (1978) *Int. J. Vitam. Nutr. Res.* **48**, 38–43.
12. Kartha, V. N. R. & Krishnamurthy, S. (1977) *Int. J. Vitam. Nutr. Res.* **47**, 394–401.
13. Krinsky, N. I. (1971) in *Carotenoids*, eds. Isler, O., Gutmann, H. & Solms, U. (Birkhaeuser, Basel, Switzerland), pp. 669–716.
14. Krinsky, N. I. (1982) in *The Science of Photomedicine*, eds. Regan, J. D. & Parrish, J. A. (Plenum, New York), pp. 397–405.
15. Simpson, K. L. & Chichester, C. O. (1981) *Annu. Rev. Nutr.* **1**, 351–374.
16. Goodwin, T. W. (1954) *Carotenoids* (Chemistry Publishing, New York).
17. Goodwin, T. W. (1962) in *Comparative Biochemistry*, eds. Florkin, M. & Mason, S. (Academic, New York), pp. 643–675.
18. Isler, O., Gutmann, H. & Solms, U., eds., (1971) *Carotenoids* (Birkhaeuser, Basel, Switzerland).
19. Foote, C. S., Chang, Y. C. & Denny, R. W. (1970) *J. Am. Chem. Soc.* **92**, 5216–5219.
20. Mathews-Roth, M. M. (1982) *J. Natl. Cancer Inst.* **69**, 279–285.
21. Santamaria, L., Bianchi, A., Arnaboldi, A., Anderoni, L. & Bermond, P. (1983) *Experientia* **39**, 1043–1045.
22. Frankel, E. N. (1980) in *Autoxidation in Food and Biological Systems*, eds. Simic, M. G. & Karel, M. (Plenum, New York), pp. 141–170.
23. Krinsky, N. I. & Deneke, S. M. (1982) *J. Natl. Cancer Inst.* **69**, 205–210.
24. Kanner, J. & Kinsella, J. E. (1983) *Lipids* **18**, 198–203.
25. Burton, G. W. & Ingold, K. U. (1984) *Science* **224**, 569–573.
26. Cutler, R. G. (1982) in *Testing the Theories of Aging*, eds. Adelman, R. G. & Roth, G. S. (CRC, Boca Raton, FL), pp. 25–114.
27. Cutler, R. G. (1984) in *Aging and Cell Function*, ed. Johnson, J. E. (Plenum, New York), pp. 1–147.
28. Cutler, R. G. (1984) in *Free Radicals in Biology*, ed. Pryor, W. A. (Academic, New York), Vol. 6, pp. 371–428.
29. Totter, J. R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1763–1767.
30. Tolmasoff, J. M., Ono, T. & Cutler, R. G. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2777–2781.
31. Jones, M. L. (1968) *Int. Zoo. Yearb.* **8**, 183–192.
32. Bowden, D. M. & Jones, M. L. (1979) in *Aging in Nonhuman Primates*, ed. Bowden, D. M. (Van Nostrand-Reinhold, New York), pp. 1–13.
33. Cutler, R. G. (1983) *Gerontology* **29**, 113–120.
34. Kahan, J. (1966) *Scand. J. Clin. Lab. Invest.* **18**, 679–690.
35. Brubacher, G. & Vuilleumier, J. P. (1974) in *Clinical Biochemistry Principles and Methods*, eds. Curtius, H. Ch. & Roth, M. (de Gruyter, New York), Vol. 2, pp. 975–982.
36. McLaren, D. S., Read, W. W. C., Awder, Z. L. & Tchalian, M. (1967) *Methods Biochem. Anal.* **15**, 1–23.
37. Folch, J., Lees, M. & Stanley, G. H. S. (1957) *J. Biol. Chem.* **226**, 497–509.
38. Lietner, Z. A., Moore, T. & Sharman, I. M. (1960) *Br. J. Nutr.* **14**, 157–169.
39. Olson, J. A. & Hayaishi, O. (1965) *Proc. Natl. Acad. Sci. USA* **54**, 1364–1370.
40. Cutler, R. G. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4664–4668.