Uptake and cleavage of β-carotene by cultures of rat small intestinal cells and human lung fibroblasts

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The purpose of the experiments reported here was to study the uptake kinetics of beta-carotene (BC) into rat small intestinal cells (hBRIE 380) in culture and the cleavage kinetics of BC to determine which of these two is the limiting process. Time and concentration dependence of uptake of BC into hBRIE 380 showed no saturation up to 25 μmol/L BC; uptake was barely reduced at 4° C even though conversion to retinol was virtually completely inhibited at that temperature. No evidence of a membrane receptor could be observed. Similar uptake kinetics, with a smaller amount taken up, were found with human lung fibroblasts (strains WI-38 and HLF). Cleavage to retinol (17.3% of BC per 10^6 cells in 24 hr) and retinoic acid (RA) (5.3%) were observed, with an apparent K_M of 9 μmol/L with respect to retinol. Both retinol and RA were identified by high-pressure liquid chromatography and derivatization. No retinyl esters were detectable under our experimental conditions. Conversion in WI-38 cells was also observed (7.8% retinol, 2.5% RA). We conclude that uptake of BC into rat hBRIE 380 cells and fibroblasts is passive and unregulated; conversion to retinol and RA is regulated, presumably through the cleavage enzyme(s).

Keywords: Beta-carotene; small intestinal cells; fibroblasts; retinol; retinoic acid

Introduction

Beta-carotene (BC) has recently assumed importance as an anti-carcinogen, both in epidemiologic studies in human[1] and in animal models.[2] The question whether BC itself is the anti-carcinogen or is first converted to retinol or retinoic acid (RA) is still controversial. Recent publications by Pung et al.[3] and Rundhaug et al.[4] show definitively that in the fibroblastic cell line C3H/10T1/2, BC and canthaxanthin inhibit chemically and physically-induced transformation without being converted to retinol, retinal, or RA.

Another important property of BC, which has been known for a long time,[5] is its lack of toxicity in contrast to retinol: the conversion of BC to retinol, when increasing concentrations of BC are given to rats, levels off before the retinol produced becomes toxic. However, the question of which is the control point that regulates the cleavage has not been established. Indeed, controversy even surrounds the clevage reaction: although the central cleavage of BC, resulting in two molecules of retinal, had been firmly established by Olson[6] and Goodman et al.,[7] and recently confirmed by Lakshman et al.,[8] this was disputed by Hansen and Maret.[9] Recently, it was reported by Napoli and Race[10] that retinal is not a free intermediate in the cleavage reaction in a cell-free system of intestinal mucosa.

In a careful study of liver storage of vitamin A in rats and chicks fed BC, Brubacher and Weiser[11] report that, for the rat, "in the range of about one to a maximum of 10 times the daily vitamin A requirement, BC is completely absorbed and transformed to vitamin A, with a relationship of one molecule BC corresponding to one molecule of retinol. With a higher intake, the log of the absorption or transformation rate decreases linearly, inverse to the log of intake." They postulate two possible mechanisms of regulation: (a) the transport of BC into the mucosal cell is regulated so that the excess is excreted in the feces; (b) the transport of BC into the mucosal cell is not limited and it is the cleavage reaction that is regulated. In the rat, only

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small amounts of BC enter the bloodstream;[12] in the mechanism (b), therefore, the unconverted BC must be re-excreted into the gut. Mechanism (b) seems the more likely in view of the work of Hollander and Ruble[13] that showed that, in the rat, BC enters the intestinal mucosa by passive diffusion.

In an attempt to distinguish between the two hypotheses above, we examined the uptake kinetics of BC into a line of small intestinal cells in culture to find out whether uptake into these cells is regulated. At the same time, we studied cleavage kinetics to determine whether BC cleavage is limiting. No attempt was made here to investigate the mechanism of cleavage.

Although whole rats[13] as well as cell-free systems[10] have been explored with respect to some of the above parameters, no studies to our knowledge have been reported with cell culture systems with cells from the small intestine, the locus of BC uptake.[14] This was made possible by the availability of an immortalized line of polarized rat SIC (hBRIE 380).[15]

Materials and methods

[^{14}C]-Beta-carotene and canthaxanthin were generously provided by Hoffmann-LaRoche Co., Nutley, NJ, USA. [^{14}C]-Beta-carotene (specific activity, 53.3 μCi/μmol) was purified (98%) through a reverse-phase high-pressure liquid chromotography (HPLC) column with methanol plus 0.5% ammonium acetate: toluene (7:3, elution volume 17 mL). Unlabeled β-carotene was purchased from Fluka, Buchs, Switzerland. Retinol, retinoic acid, retinyl palmitate, and lycopene were all purchased from Sigma Co., St. Louis, MO, USA. Tetrahydrofuran (THF), 99.9%, and Diazald for diazomethane preparation were purchased from Aldrich Chemical Co., Milwaukee, WI, USA. Dimethylsulfoxide (DMSO) was purchased from Baxter Co., Muskegon, MI, USA. All HPLC solvents were optimum grade from Fischer Scientific, Kent, WA, USA.

Cell culture

Human lung fibroblasts (W1-38) and human embryonic lung fibroblasts (HLF) were purchased from American Type Culture Collection, Rockville, MD, USA, and maintained in Earl's Salt Minimum Essential Medium supplemented with 10% heat-inactivated fetal bovine serum, sodium bicarbonate (2.2 g/L), 100 units/mL penicillin, 0.1 mg/ml streptomycin, 0.1% lactalbumin, and 2 mmol/L L-glutamine. hBRIE 380 cells were maintained in Iscove's Modified Dulbecco's Medium with 10% fetal bovine serum and sodium bicarbonate (3.04 g/L). The hBRIE 380 cell line is a hybrid intestinal epithelial cell line derived from the fusion of a spontaneously transformed rat small intestinal mucosal epithelial cell line with freshly isolated mucosal epithelial cells from the rat duodenum.[15]

Cells were grown in Corning (Corning Glass Works, Corning, NY, USA) dishes (35 mm diameter) in humidified atmosphere of air/CO_2 (95:5) at 37° C.

HPLC

HPLC was performed with a Beckman/Altex model (Beckman Instruments, Fullerton, CA, USA) with reverse-phase column (0.46 × 15 cm Beckman Ultrasphere ODS, 5 μm particles). The sample was eluted at a flow rate of 1.5 mL/min. with a linear gradient developed in 12 min from 100% solvent A (methanol plus 0.5% ammonium acetate) to 80% solvent A plus 20% toluene and monitored at 452 nm for the separation of carotenoids. The separation of retinoids was carried out at a flow rate of 1.5 mL/min. with a mixture of water plus 2% ammonium acetate/acetonitrile (28:72)[9] (unless otherwise stated).

[^{14}C]-β-Carotene–addition to medium

The method for delivering carotenoids in a water solution was adapted from that of Bertram et al.[16] The micellar-like nature of this "solution" has been demonstrated by Bertram et al[16] who suggested that when BC is "solubilized" by the THF in water, it exists in a highly constrained, although not solid state. Five to 10 μL of a solution of BC in THF:DMSO (1:1) (0.5–3 mmol/L) were rapidly injected into 1 mL of culture medium to give a final concentration varying over a range of 1 μmol/L to 28 μmol/L, and stirred for 30 min. This BC-containing medium was then incubated with cells previously grown to confluency.

Analysis of β-carotene uptake and cleavage

After incubating with [^{14}C]-β-carotene at different concentrations for different times, the medium was removed and the cell monolayer rinsed three times with phosphate buffer saline (PBS) (until no longer radioactive). The cells were then detached with 0.25% trypsin, pelleted, and resuspended in 0.4 mL of PBS:ethanol (1:1) with 0.025% butylated hydroxy-toluene (BHT). The cell suspension was then sonicated for a total of 30 sec in three bursts of 10 sec. each, keeping it in ice, by an ultrasonic sonifier model Ultrasonics W 185. Lycopene (5 μL, 0.5 mmol/L) in THF was added to the sample before extraction as internal standard. Calculations were made on the basis of the percent of recovery of the internal standard. The suspension was saturated with NaCl and extracted twice with 0.6 mL of hexane plus 0.025% BHT. When retinoid analysis was carried out, 5 μL of retinol (1 mmol/L) and 5 μL of retinoic acid (1 mmol/L) in chloroform were added as internal standards. The hexane phase was evaporated to dryness under N_2, reconstituted in the HPLC solvent, and injected into the HPLC column.

Each fraction was collected from the column and mixed with 10 mL of Cytoscint (ICN, Irvine, CA, USA) scintillation liquid, and the radioactivity was measured by a liquid scintillation counter.

Chemical identification of retinol and retinoic acid

The acetylation of retinol was done using acetyl chloride according to Fischli et al.[17] The reaction products were detected by HPLC with methanol plus 0.5% ammonium acetate at a flow rate of 1 mL/min followed by radioactivity measured for each HPLC fraction by liquid scintillation counting. The methylation of retinoic acid was performed by mixing diazomethane in ether with the appropriate HPLC fraction in ether as a reagent and monitoring the reaction product by HPLC with acetonitrile: water plus 2% ammonium acetate (78:28) at a flow rate of 1.5 mL/min and liquid scintillation counting as described above.

Results and discussion

Uptake of beta-carotene

Concentration and time-dependence of uptake of BC into hBRIE 380 cells is shown in *Figure 1*. The absence of a receptor-regulated uptake into these cells

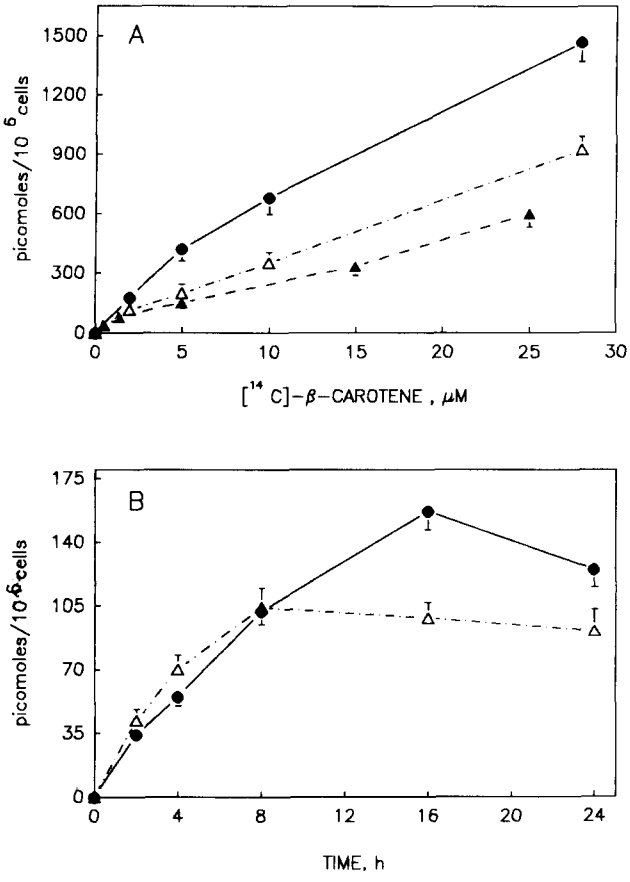

Figure 1 Time and concentration dependence of uptake of [^{14}C]-beta-carotene by small intestinal cells and human lung fibroblast. A: Different concentrations of [^{14}C]-BC in THF:DMSO (1:1) were rapidly mixed with culture medium as described in Materials and methods and incubated for 8 hr with confluent fibroblasts, and 16 hr with confluent hBRIE 380 cells at 37° C. B: [^{14}C]-BC (2 nmol) were mixed with 1 mL of culture medium and incubated with confluent hBRIE 380 at 37° C. At each concentration (A) and time (B) the cells were washed, collected, extracted, and analyzed for [^{14}C]-BC content as described in Materials and methods. Values are the mean of three determinations ± SE. Closed circles, hBRIE 380; open triangles, WI 38; closed triangles, HLF.

is suggested because there is no leveling-off of uptake up to a concentration of 25 μmol/L BC. Higher concentrations could not be tested because of the insolubility of BC. Unincorporated excess BC in the medium was completely removable by washing, because of the total water miscibility of the THF-DMSO solution of BC and the absence of any microscopically detectable

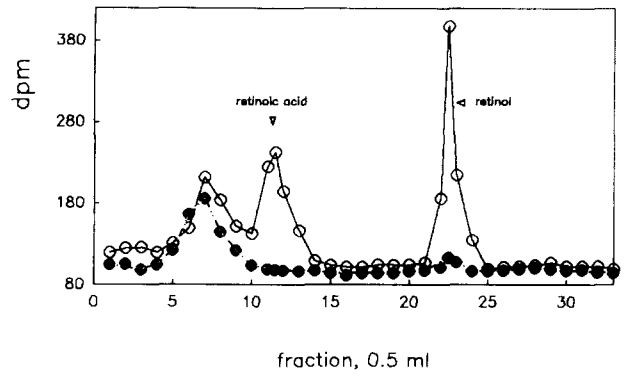

Figure 2 HPLC analysis of [^{14}C]-Retinol and [^{14}C]-Retinoic Acid produced from [^{14}C]-BC by cells. [^{14}C]-BC (1 nmol) were mixed with 1 mL of culture medium and incubated in presence (○) or in absence (●) of confluent hBRIE 380 at 37° C. After 24 hr the cells or aliquot of the medium were analyzed for BC cleavage products as described in Materials and methods.

crystalline BC. After washing three times with PBS no radioactivity could be detected in the washes (data not shown).

The temperature-dependent splitting of BC to retinol and retinoic acid and the complete absence of extracellular conversion activity (no retinol or retinoic acid could be detected in the cell culture medium after 24 hr incubation with or without cells, *Figure 2*), further suggested not only that BC is incorporated but is also available for an enzymatic activity that accounts for its conversion.

The slight inhibition of BC uptake at 4° C (20%; *Table 1*), especially when compared with inhibition of the conversion of BC to retinol (80%; *Table 1*), seems to exclude uptake as an enzymatic or receptor-mediated process. The standard experiment to demonstrate receptor saturation, by incubation of ^{14}C-BC with an excess of unlabeled BC, also failed to show receptor-regulated uptake (*Table 2*). Similarly, the carotenoid closely related to BC, canthaxanthin, did not inhibit uptake (*Table 2*). Because with excess unlabeled BC much more total BC entered the cells, the cleavage enzymes(s) were presumably saturated and hence a smaller amount of labeled cleavage products was found (*Table 2*).

Concentration dependence of uptake of BC into two different cell lines of human lung fibroblasts (WI-38, fetal, and HLF) (*Figure 1*) was similar to that into

Table 1 Effect of temperature on [^{14}C]-beta-carotene uptake and conversion to retinol in small intestinal cells (hBRIE 380)

Temperature (C)	Treatment Incubation time	[^{14}C]-BC uptake	[^{14}C]-retinol formation
		picomol/10^6 cells*	picomol/10^6 cells*
37°	4 hr	61 ± 5.2	5.9 ± 1.1
4°	4 hr	51 ± 4.5	1.1 ± 0.21
37°	24 hr	119 ± 11.3	18.9 ± 2.1
4°	24 hr	95 ± 7.3	2.1 ± 0.33

* Mean and SEM of 3 incubations.

Table 2 Effect of unlabeled carotenoids on [^{14}C]-beta-carotene uptake and conversion to retinol and retinoic acid in small intestinal cells (hBRIE 380)

Incubations with	[^{14}C]-BC	[^{14}C]Retinol	[^{14}C]Retinoic Acid
	picomol/10^6 cells		
1 μmol/L [^{14}C]-BC	63 ± 6.0*	6.1 ± 0.7	3.4 ± 0.2
1 μmol/L [^{14}C]-BC plus 20 μmol/L unlabeled BC	60 ± 5.4	3.2 ± 0.3	1.4 ± 0.2
1 μmol/L [^{14}C]-BC plus 20 μmol/L Canthaxanthin	65 ± 4.9	6.2 ± 1.1	2.9 ± 0.3

* Mean and SEM of 3 incubations.

hBRIE 380, except that only about half the amount was taken up at the lower concentrations. Time dependence of WI-38 was similar to that found in hBRIE 380.

The mechanism of uptake of BC into hBRIE 380 and fibroblasts seems to be passive and confirms the observation of Hollander and Ruble[13] with rat intestinal loops in vivo. Uptake of BC into cells from the medium at a comparable medium concentration of BC to that of Rundhaug et al.[4] was less than one-tenth as great as reported by these authors, also using fibroblast cells. Possibly the use of water-dispersible beadlets by these authors could account for the difference. We used a micellar suspension (micellar particle diameter <0.45 micrometer). Rundhaug et al.[4] detected a small loss (<4%) of medium BC by chemical oxidation in 24 hr; in our system, BC was stable over 24 hr.

Cleavage of beta-carotene

As shown in *Figure 3*, hBRIE 380 cells produce both retinol and retinoic acid (RA) from BC: time dependence is shown in *Figure 3A* and concentration dependence (for retinol) in *Figure 3B*. The production of retinol observed in 24 hr was 17.3% of the BC in the cells, of RA, 5.35%, at a BC concentration of 1 μmol/L with 1 × 10^6 cells. The apparent K_M was 9 μmol/L with respect to retinol formation, of the same order of magnitude as that found by Napoli and Race[10] for their cell-free system (20.6 μmol/L). *Table 2* shows that, as expected, excess unlabeled BC inhibited the conversion of ^{14}C-BC to labeled retinoids, whereas the closely-related carotenoid canthaxanthin did not. Thus, conversion was appreciable, with 22% of the BC converted to retinol and RA in 24 hr by 1 × 10^6 hBrie 380 cells.

Human lung fibroblasts (WI-38) or HLF also converted BC to retinol and RA (time dependence, *Figure 4A;* concentration dependence for retinol formation, *Figure 4B*), but at a rate about one-half that of hBRIE 380 (7.8% retinol and 2.5% RA at a BC concentration of 1 μmol/L by 1 × 10^6 cells). It was not possible to determine a K_M for retinol formation with these cells, since the maximum achievable concentration of BC in the medium was 25 μmol/L, at which the conversion to retinol had not yet leveled-off.

Identification of retinol and RA (*Figures 5 and 6*) was by retention times on HPLC compared with authentic samples in two solvent systems (water:ammonium acetate: acetonitrile; methanol-toluene), and by conversion of retinol to its acetate and RA to its methyl ester, with shifts in retention times corresponding to those observed for light absorption at 326 and 350 nm, respectively.

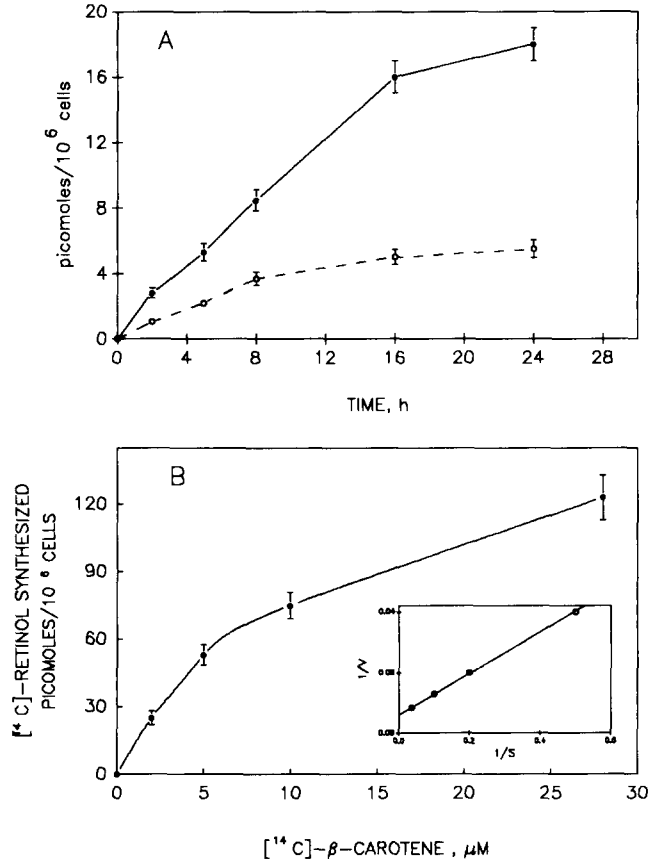

Figure 3 Time and concentration dependence of the conversion of [^{14}C]-beta-carotene to retinol and retinoic acid by small intestinal cells. A: Retinol (solid line) and retinoic acid (broken line) synthesis was analyzed at various times of incubation in presence of a fixed amount of [^{14}C]-BC (2 [μmol/L) as described in Materials and methods. B: increasing concentrations of [^{14}C]-beta-carotene were incubated with cells for 16 hr as described in *Figure 1A*. The inset represents the linear transformation of the saturation curve of retinol synthesis. The HPLC analyses were performed as described in Materials and methods. Values are the mean of three determinations ± SE.

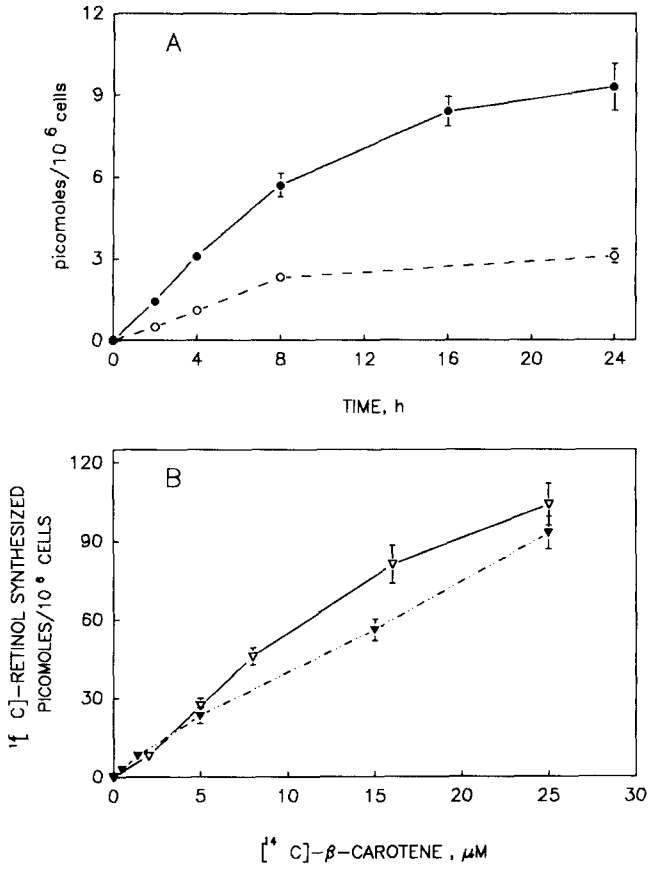

Figure 4 Time and concentration dependence of the conversion of [^{14}C]-beta-carotene to retinol and retinoic acid by human lung fibroblasts. A: Retinol (solid line) and retinoic acid (broken line) synthesis were analyzed at various times of incubation in presence of a fixed amount of [^{14}C]-β-carotene (2 μmol/L) as described in Materials and methods. B: Increasing concentrations of [^{14}C]-β-carotene were incubated with cells (WI-38, open triangles; HLF, closed triangles) for 16 hr as described for *Figure 1A*. The HPLC analyses were performed as described in Materials and methods. Each point represents the mean ± SEM (n = 6).

A more polar-labeled peak than either retinol or RA (*Figures 5 and 6*) was detected by incubation of ^{14}C-BC, which was not hydrolyzed by acid or alkali to RA or retinol, and therefore was not retinoyl glucuronide.[18] It was not acetylated by acetylchloride nor methylated by diazomethane.

The temperature-dependent formation of retinol (*Table 1*), together with the saturation curve (*Figure 3B*), clearly indicate an enzymatic process, particularly in absence of conversion without cells. Human lung fibroblasts converted about 10% BC to retinol and retinoic acid in 24 hr. This is in contrast to the findings of Rundhaug et al.[4] who could not detect cleavage of BC in their fibroblastic cell system (C3H10T1/2), finding neither retinol nor retinal. This discrepancy may be due to the use of water-soluble BC beads or a different cell line by these authors.

The absence of detectable retinyl esters was surprising, in view of the results in vivo of Olson.[19] This could be explained by the low rate of conversion of the retinol produced in the cleavage reaction to esters in our cell system. As shown in *Table 3*, when these cells were exposed to labeled retinol, only about 3.5%–6.7% of the entering retinol was esterified in 2 hr, so that the esterification of the retinol produced by BC cleavage would be undetectable in our system. Furthermore, as shown by El-Gorab et al.,[20] esterification is strictly dependent on the presence of bile salts, which were not used in the present work.

We could detect no retinal or retinyl esters in hBRIE 380 in spite of the fact that the limit of detection of our experimental system was less than one picomole. The failure to detect any retinal is in agreement both with the fact that the intracellular concentration of this retinoid was found to be close to zero in different cell types (with the exception of the visual cells): and with the finding by Napoli and Race[10] of absence of free retinal in a cell-free system of rat small intestine that converts BC to retinoic acid. Lakshman et al.,[8] on the other hand, show retinal to be an intermediate in the conversion of BC to retinol in rat intestinal mucosa. Nevertheless, as the same authors suggest, it is necessary to fractionate the BC cleavage enzyme activity from other interfering activities that could carry out the rapid metabolic conversion of the retinal intermediate. Therefore in our cell system, the occurrence of retinal might be transient.

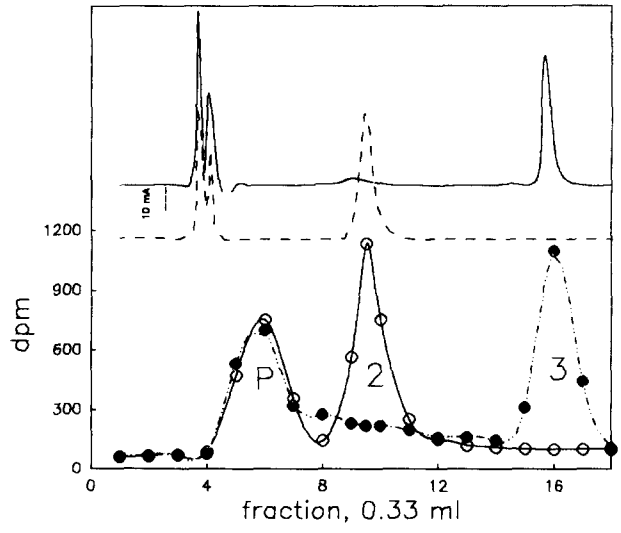

Figure 5 HPLC analysis of retinyl ester produced after acetylation of the retinol formed by beta-carotene cleavage in small intestinal cells. [^{14}C]-BC was incubated with hBRIE 380 for 16 hr. After collecting the cells, 5 μL of authentic retinol (2.5 nmol) in THF were added and the cell suspension was sonicated and extracted. The hexane phase was evaporated by dryness under N_2 and the samples were reconstituted in chloroform. The acetylation was performed as described in the published procedure.[17] The HPLC solvent system used as methanol plus 0.5% ammonium acetate at a flow rate of 1 mL/min. Measurements of optical density were by flow detector at 330 nm. The HPLC profile of acetylated (closed circle) and non-acetylated (open circle) [^{14}C]-retinol synthesized from [^{14}C]-BC in hBRIE 380 is shown in the lower plot. Peak P: unidentified polar compounds. Peak 2: retinol; Peak 3: retinyl acetate. The upper traces are the absorbance signal at 350 nm.

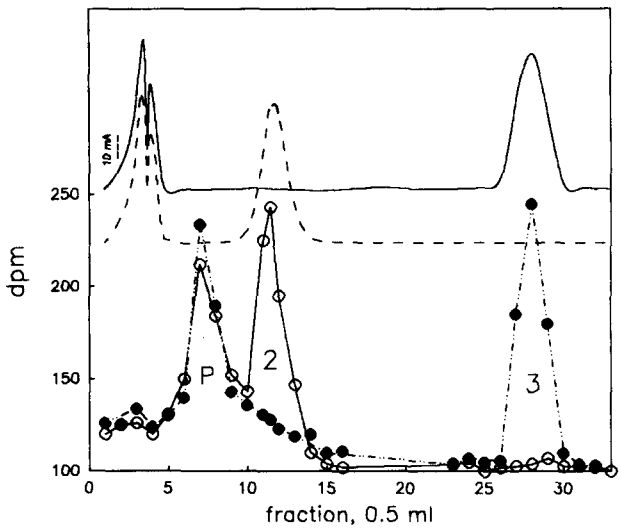

Figure 6 HPLC analysis of methyl ester produced after methylation by diazomethane of retinoic acid formed by conversion of beta-carotene in SIC. [^{14}C]-BC (2 μmol/L) was incubated with SIC for 6 hr as described in Materials and methods. After collecting the cells, 5 μL of authentic retinoic acid (2.5 nmol) in THF were added and the cell suspension was sonicated and extracted with hexane. An ether solution of diazomethane was added dropwise to the hexane phase and the mixture was stirred in ice for 15 min before adding acetic acid. After evaporating to dryness and resuspending in the HPLC solvent system, the samples were analyzed by HPLC as described in Materials and methods. Measurement of optical density was by flow detection at 350 nm. The HPLC profile of methylated (closed circles) and non-methylated (open circles) labeled retinoic acid synthesized from [^{14}C]-BC by hBRIE 380 is shown. Peak P; unidentified polar compound; Peak 2, retinoic acid; Peak 3, methyl retinoate. Upper traces: absorbance at 350 nm.

Table 3 Retinol uptake and esterification in hBRIE 380 cells

Incubation time (hours)	Retinol (picomol/10^6 cells)	Ester (picomol/10^6 cells)
2	139 ± 7.3	4.9 ± 0.5
4	145 ± 10.2	7.6 ± 1.0
6	120 ± 6.0	8.5 ± 0.6

Abbreviations

BC	beta-carotene
RA	retinoic acid
THF	tetrahydrofuran
DMSO	dimethyl sulfoxide
HPLC	high-pressure liquid chromatography
BHT	butylated hydroxy-toluene

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References

1 Hennekens, C.H., Mayrent, S.L., and Willet, W. (1986). Vitamin A, carotenoids and retinoids. *Cancer*, **58**, 1837–1841
2 Moon, R.C. (1989). Comparative aspects of carotenoids and retinoids as chemopreventive agents for cancer. *J. Nutr.* **119**, 127–134
3 Pung, A., Rundhaug, J.E., Yoshizawa, C.N., and Bertram, J.S. (1988). Beta-carotene and canthaxanthin inhibit chemically and physically-induced neoplastic transformation in 10T1/2 cells. *Carcinogenesis* **9**, 1533–1539
4 Rundhaug, J.E., Pung, A., Read, C.M., and Bertram, J.S. (1988). Uptake and metabolism of beta-carotene and retinol by C3H/10T1/2 cells. *Carcinogenesis* **9**, 1541–1545
5 Moore, T. (1957). *Vitamin A*. Elsevier Publishing Co., New York, NY
6 Olson, J.A. and Hayaishi, O. (1965). The enzymatic cleavage of beta-carotene into vitamin A by soluble enzymes of rat liver and intestine. *Proc. Natl. Acad. Sci. USA* **54**, 1364–1370
7 Goodman, D.S., Huang, H.S., and Shiratori, T. (1966). Mechanisms of the biosynthesis of vitamin A from beta-carotene. *J. Biol. Chem.* **241**, 1929–1932
8 Lakshman, M.R., Mychkovksy, I., and Attlesey, M. (1989). Enzymatic conversion of all-*trans*-beta-carotene to retinal by a cytosolic enzyme from rabbit and rat intestinal mucosa. *Proc. Natl. Acad. Sci. USA* **89**, 9124–9128
9 Hansen, S. and Maret, W. (1988). Retinal is not formed in vitro by enzymatic cleavage of beta-carotene. *Biochem.* **27**, 200–206
10 Napoli, J.L. and Race, K.R. (1988). Biogenesis of retinoic acid from beta-carotene. *J. Biol. Chem.* **257**, 13385–13389
11 Brubacher, G.B. and Weiser, H. (1985). The vitamin A activity of beta-carotene. *Internat. J. Vit. Nutr. Res.* **55**, 5–15
12 Shapiro, S.S., Mott, D.J., and Machlin, L.J. (1984). Kinetic characteristics of beta-carotene uptake and depletion in rat tissue. *J. Nutr.* **114**, 19024–19033
13 Hollander, D. and Ruble, P.E. (1978). Beta-carotene intestinal absorption: bile, fatty acid, pH and flow rate effects on transport. *Amer. J. Physiol.* **233**, E686–E691
14 Huang, H.S. and Goodman, D.S. (1965). Vitamin A and carotenoids. *J. Biol. Chem.* **240**, 2839–2844
15 Aponte, G.W., Keddie, A., Hallden, G., Hess, R., and Link, P. (1991). Polarized intestinal hybrid cell lines derived from primary culture: establishment and characterization. *Proc. Natl. Acad. Sci.* V.5. **88**, 5282–5286
16 Bertram, J.S., Pung, A., Churley, M., Kappock, T.J., IV, Wickens, L.R., and Cooney, R.V. (1991). Diverse carotenoids protect from chemically-induced neoplastic transformation. *Carcinogenesis* **12**, 671–678
17 Fischli, A., Mayer, H., Simon, W., and Stoller, H.J. (1976). A synthesis of vitamin A according to the sulfone method. *Helv. Chim. Acta* **59**, 397–405
18 Barua, A.B. and J.A. Olson. (1986). Retinoyl beta-glucuronide, an endogenous compound of human blood. *Amer. J. Clin. Nutr.* **43**, 481–485
19 Olson, J.A. (1961). The conversion of radioactive beta-carotene into vitamin A by rat intestine in vivo. *J. Biol. Chem.* **236**, 349–356
20 El-Gorab, M.I., Underwood, B.A., and J.D. Loerch. (1975). The roles of bile salts in the uptake of β-carotene and retinol by rat everted gut sacks. *Biochim. Biophys. Acta* **401**, 265–277