

## Uptake and Distribution of Carotenoids, Retinol, and Tocopherols in Human Colonic Epithelial Cells *in Vivo*

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### Abstract

Studies suggest that micronutrients such as the tocopherols, retinol, and the carotenoids have a chemopreventive action against colonic carcinogenesis and that they may be essential for the functioning and structural integrity of the gastrointestinal epithelium. In this study, we have determined the concentrations of tocopherols, retinol, and the carotenoids in human colonic epithelial cells using a noninvasive procedure developed in this laboratory (G. P. Albaugh *et al.*, *Int. J. Cancer*, 52: 347-350, 1992). In subjects on a normal diet, almost all of these micronutrients were restricted to cells in the density range of  $\rho 1.065-1.090$  and  $\rho 1.090-1.110$ . The lighter fraction ( $\rho 1.033-1.064$ ), representing the most senescent subpopulation, retained these micronutrients only when the subjects were on diets rich in vegetables. Cells isolated from subjects on their usual diets gave the following values expressed as ng/10<sup>7</sup> cells:  $\alpha$ -tocopherol, 93-151;  $\gamma$ -tocopherol, 152-280; retinol, 12-20; lutein, 4-18; cryptoxanthin, not detected; lycopene, 0-17;  $\alpha$ -carotene, 3-7; and  $\beta$ -carotene, 6-9. Peak responses in specific micronutrients following 5 days on a high carotenoid diet showed a lag period of at least 5 days, corresponding to the turnover rates of the epithelium itself. The evidence suggests that uptake of these micronutrients by the colonic mucosa occurs in the deep cryptal zone where the actively proliferating cells extract the nutrients from the systemic circulation.

### Introduction

Recent studies on micronutrients such as the tocopherols, retinoids, and the carotenoids have demonstrated the potential use of these substances as agents for the chemoprevention of certain

cancers (1-4). Since they may be essential for the functioning and structural integrity of the gastrointestinal epithelium, they may also have a role in the chemoprevention of colonic cancer. We, therefore, initiated a study to examine the *in vivo* distribution of these micronutrients in human colonic epithelial cells. These studies were made possible with the advent of a novel noninvasive technique, developed in our laboratories, for the isolation of colonic epithelial cells (5, 6). In this report, we present evidence to demonstrate that: (a) the presence of these micronutrients in colonic epithelial cells; and (b) the kinetics of uptake of these micronutrients parallel the kinetics and turnover of the colonic epithelium.

### Materials and Methods

**Subjects.** In conformity with the Code of Federal Regulations, ethical clearance of the research protocol was unnecessary under section 101(b), paragraph 4 of the U.S. Department of Agriculture Directive 605.1. Volunteers from the laboratory provided stool samples from which colonic epithelial cells were isolated. In a feeding experiment, three of the investigators volunteered to consume for 5 consecutive days approximately 100 g/day of one of the following well-cooked (boiled) vegetables, kale, tomatoes or spinach, as sources rich in lutein, lycopene, or  $\beta$ -carotene, respectively. These subjects provided stool samples at various time intervals following the dietary intervention.

**Isolation and Fractionation of Cells.** Colonic epithelial cells were isolated from freshly passed stools by procedures described earlier (6). The general procedure is outlined in Fig. 1. About 5-10 g of stools were placed in 25 ml of the transport medium in a 50-ml screw-capped centrifuge tube. The sample was dispersed by gentle inversion several times before sending it to the laboratory for processing. The stool suspension was further diluted with an additional 225 ml of dispersing medium and gently dispersed in a Stomacher (Tekmar, Cincinnati, OH) for 30 s. The suspension was centrifuged at  $900 \times g$  for 10 min, and the pellet was dispersed in 60 ml of dispersing medium. A preliminary purification was carried out by centrifugation over Histopaque 1119 (Sigma Chemical Co., St. Louis, MO), and the cells above the interface were recovered and further purified by centrifugation through a Percoll (Pharmacia Biotech, Inc., Uppsala, Sweden) linear density gradient (6). The density gradient is diagrammatically represented in Fig. 2. The cells were dispersed over the density range of  $\rho 1.033-1.120$ , and they were fractionated into three subpopulations ( $\rho 1.033-1.064$ ,  $\rho 1.064-1.090$ , and  $\rho 1.090-1.111$ ); the least dense ( $\rho 1.033-1.064$ ) was the most senescent subpopulation.

**Analysis of Cells for Micronutrients.** The cell pellets containing a predetermined number of cells were stored at  $-85^\circ\text{C}$  and protected from light by a covering of aluminum foil. The specimens were thawed and centrifuged for 8 min at  $12,000 \times g$  at  $4^\circ\text{C}$  to obtain a compact cell pellet. The supernatant was removed and replaced with 300  $\mu\text{l}$  of ethanol containing ascor-

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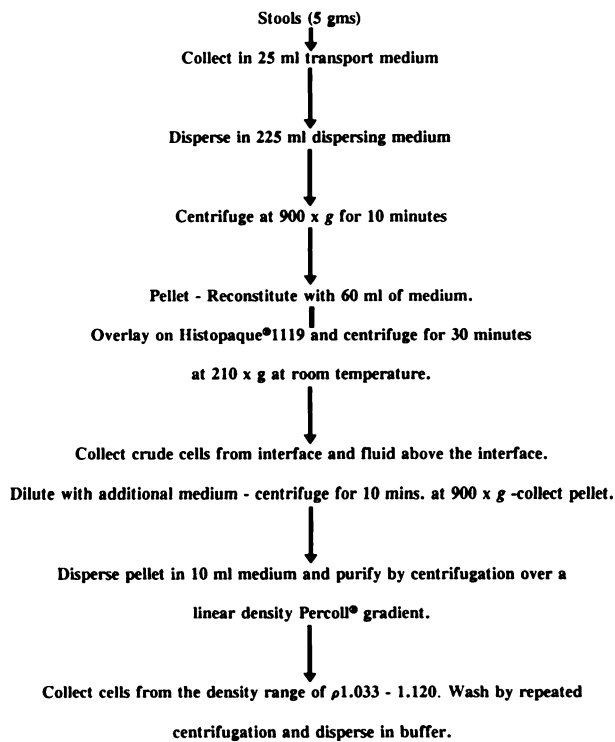


Fig. 1. Flow chart for isolation of colonic epithelium.

bic acid (1 g/100 ml) and butylated hydroxytoluene (10 mg/100 ml).

The sample vials were capped and vortexed for 30 s at room temperature, followed by disruption of the cell pellet by sonication at 30°C for 10 min. The entire cell pellet was saponified in an ethanolic solution for 20 min at 78°C as described earlier (7). Samples were cooled in an ice bath and extracted immediately, twice, with hexane-containing butylated hydroxytoluene. The entire extraction procedure was carried out under yellow light. The hexane extracts were combined and evaporated under nitrogen, followed by reconstitution in the high-performance liquid chromatography mobile phase (ethanol-acetonitrile, 1:1, containing 0.1 ml of diethylamine/liter). The micronutrients were analyzed by high-performance liquid chromatography, as described by Sowell *et al.* (8). The data are presented in Tables 1 and 2.

## Results

The data on the distribution of retinol, tocopherols, and carotenoids in subjects on a "normal" diet are shown in Table 1. The exfoliated colonic epithelial cells showed the presence of significant amounts of tocopherols, especially  $\gamma$ -tocopherol. Significant amounts of retinol were also present. In general, the carotenoid content was rather small, with cryptoxanthin undetected.

The distribution of retinol,  $\alpha$ -tocopherol, and  $\beta$ -carotene in colonic cells as a function of density is shown in Table 2. The fraction with the lowest density representing the most senescent cells has the least amount of these micronutrients, as opposed to the fraction with the highest density containing the highest concentrations of these nutrients.

The time course of carotenoid accumulation in colonic

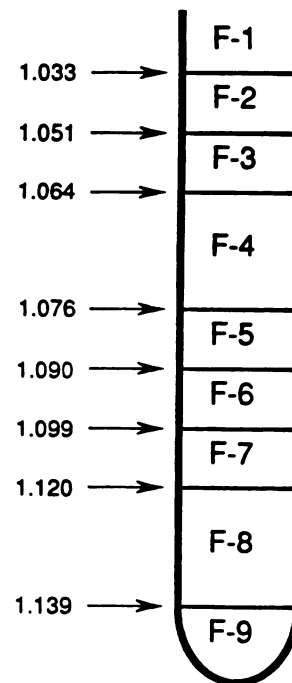


Fig. 2. Diagrammatic representation of cell fractionation on a Percoll7 linear density gradient. *Left*, the density intervals between  $\rho$ 1.033 and 1.139.

Table 1 Concentration range of micronutrients in exfoliated colonic epithelial cells<sup>a</sup>

Nutrient	Concentration ng/10 <sup>7</sup> cells
$\alpha$ -Tocopherol	93-151
$\gamma$ -Tocopherol	152-280
Retinol	12-20
Lutein	4-18
Cryptoxanthin	Not detected
Lycopene	0-17
$\alpha$ -Carotene	3-7
$\beta$ -Carotene	6-9

<sup>a</sup> These ranges were obtained on samples from four individuals.

epithelial cells is shown graphically in Figs. 3-5. For  $\beta$ -carotene (subject no. 3), the peak concentration was at day 7; for lycopene (subject no. 2), it was day 5, closely followed by day 13; and for lutein, (subject no. 1), it was day 13.

## Discussion

In this report, we have demonstrated the feasibility of studying the flux of selected micronutrients in the colonic epithelium noninvasively. Exfoliated colonic epithelial cells isolated from stools show the presence of retinol,  $\alpha$ - and  $\gamma$ -tocopherols, and a number of carotenoids. The tocopherols are in relatively large amounts (93-280 ng/10<sup>7</sup> cells), with the  $\gamma$ -isomer being in much higher concentrations than the  $\alpha$ -isomer. This is in contrast to what is normally encountered in serum and other tissues and might be a reflection of preferential extraction of the  $\gamma$  isomer by colonic epithelial cells. Mobarhan and associates (9) have studied mucosal concentrations of  $\beta$ -carotene in patients

Table 2 Distribution of micronutrients in colonic epithelial cells as a function of density<sup>a</sup>

Nutrients	Density range		
	1.033–1.064	1.064–1.090	1.090–1.11
Retinol	0–3.2% (2.25%)	7.2–30.2% (19.6%)	69–90% (78.8%)
$\alpha$ -Tocopherol	0.1–4.4% (2.5%)	5.9–32% (19.9%)	63–91% (77.5%)
$\beta$ -Carotene	0.7–5.3% (3.0%)	21.7–35.6% (28.7%)	59–100% (78.9%)

<sup>a</sup> Distributions are expressed as range %. Values within parentheses are means. The results reported here are from three subjects on samples collected on three separate occasions at intervals of 4 months.

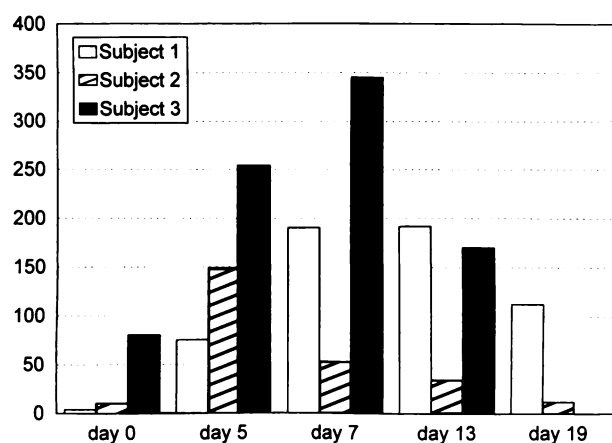


Fig. 3. Time course of uptake of  $\beta$ -carotene by colonic epithelial cells. Subject 1, kale; Subject 2, tomatoes; Subject 3, spinach. Values are nanograms per 10 million cells.

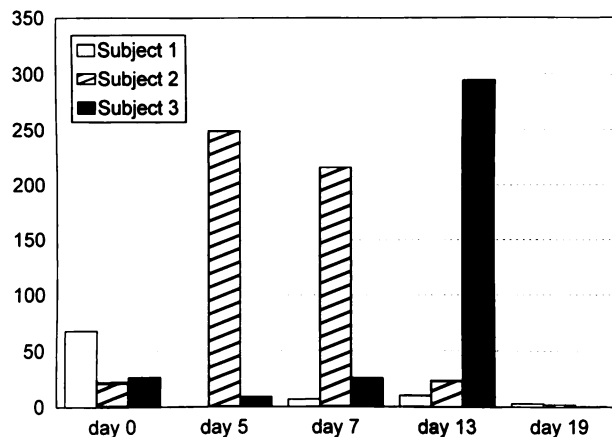


Fig. 4. Time course of uptake of lycopene by colonic epithelial cells. Subject 1, kale; Subject 2, tomatoes; Subject 3, spinach. Values are nanograms per 10 million cells.

with colonic neoplasia, and they have reported a significant decrease in  $\alpha$ -tocopherol levels in patients given  $\beta$ -carotene.

As a function of density, the highest concentrations of the micronutrients are found in cells in the fraction with the highest density ( $\rho$ 1.090-1.11). Since these are young cells compared to

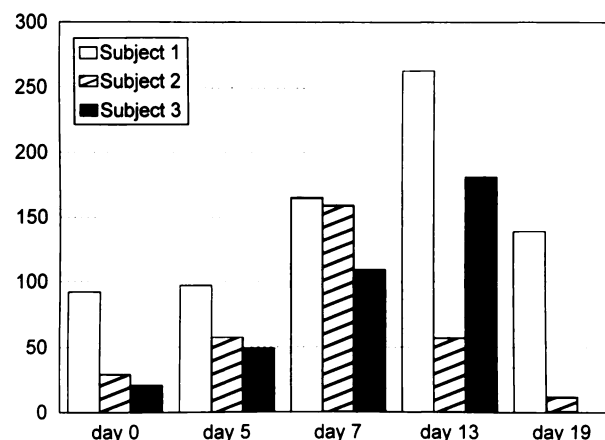


Fig. 5. Time course of uptake of lutein by colonic epithelial cells. Subject 1, kale; Subject 2, tomatoes; Subject 3, spinach. Values are nanograms per 10 million cells.

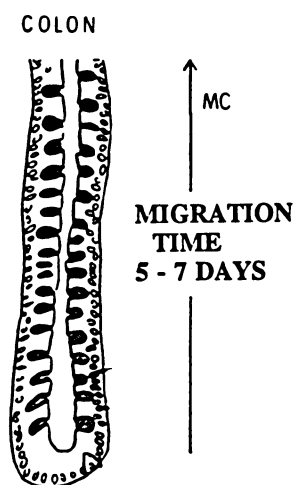


Fig. 6. Colonic crypt and the migration of colonic cells from the deep cryptal zone to the luminal surface as proposed by Lipkin (11).

cells from the lighter fractions (more senescent), it appears that the senescence of colonic epithelium is associated with depletion of these micronutrients (6).

In the study where subjects consumed the carotenoid-rich vegetables, kale (lutein, subject no. 1), tomatoes (lycopene, subject no. 2), or spinach ( $\beta$ -carotene, subject no. 3) for 5 days, there was a lag of at least 5 days before maximum response in carotenoids was seen in the colonic epithelial cells. Although the subjects followed their customary diet habits during the course of the study, there was considerable overlap in response among the three carotenoids, regardless of the nature of the food supplement. As an example, subject no. 3 who ate spinach showed significant responses in lycopene and lutein on day 13 in addition to a strong  $\beta$ -carotene response on day 7. This is attributable to the fact that the vegetable supplements contain other carotenoids, as well, in lower concentrations. The absorption of carotenoids from the diet is highly variable among different individuals, as reflected in postprandial blood responses within the first 24 h following a test dose (10). In this

regard, the time course of carotenoid uptake by colonic cells lagged behind that of the blood by 5–7 days. Although the colonic mucosa is in intimate contact with the digested food containing unabsorbed carotenoids and other micronutrients, it appears from the data that there is virtually no uptake from the luminal contents. On the other hand, it is interesting to note that the kinetics of uptake of lutein, lycopene, and  $\beta$ -carotene are similar to the turnover rates of the epithelium itself. As seen in Fig. 6, the cells from the proliferating zone migrate toward the luminal surface in about 5–7 days in humans (11), which is approximately the same as the lag time for the appearance of the nutrients in the exfoliated cells. From this observation, it would appear that the uptake and turnover of the carotenoids in the cells exfoliated from the colon are synchronous with the turnover rate of the colonic epithelium.

From this evidence, we suggest that these micronutrients have to be first absorbed, systemically, before being transferred to the proliferating cells in the deep cryptal zone. If this observation is confirmed in future studies, this technique will lend itself to the development of a methodology for studying the influence of diet and other factors on the turnover rates of the colonic epithelium.

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