Beta-carotene accumulation in serum and skin

Martin R Prince and Joan K Frisoli

ABSTRACT  The accumulation of β-carotene in serum and skin was evaluated in human volunteers. A single 51-mg dose of β-carotene given in the absence of dietary fat resulted in no detectable change in serum β-carotene. The same dose administered with 200 g fat increased serum β-carotene 2.5-fold at 40 h. Similarly, administering β-carotene daily in three divided doses with meals raised the serum β-carotene concentration three times as high compared with the same total dose administered once a day; both regimens had the same time constant for serum accumulation: 9–10 d. Remittance measurements of skin color demonstrated that the accumulation of β-carotene in skin was delayed by up to 2 wk compared with serum accumulation. These data indicate that β-carotene absorption requires dietary fat and is enhanced by administering with meals but there is a long time constant for serum (10 d) and tissue (several weeks) accumulation.

KEY WORDS  Atherosclerosis, carotenoids, laser angioplasty, nutrition, lipid, lipoprotein, pharmacokinetics

Introduction

Beta-carotene is in the carotenoid family of pigments that occur naturally in plants and are consumed by humans in the form of green and yellow vegetables. Although the function of carotenoids in plants is not completely understood, their lipophilicity and ability to quench reactive chemical species suggest that they may be important for protecting plant membranes during photosynthesis (1). In humans, carotenoids and especially β-carotene are an important source of vitamin A and are also postulated to protect human cells from the reactive species that may induce cancer and atherosclerosis (2–4). There is also recent evidence that carotenoids can induce cell-mediated tumor lysis (5). Their ability to accumulate in lipid-rich atherosclerotic plaque makes selective removal of plaque possible with laser radiation tuned to the carotenoid absorption peak (6–9). In addition, a carotenoid metabolite, retinoic acid, recently has been shown to play a fundamental role in cell differentiation and organogenesis (10).

Despite this interest in β-carotene, its absorption and tissue accumulation are not completely understood. Beta-carotene was approved for medical use as an orphan drug to treat a rare photosensitizing skin disorder, erythropoietic protoporphyria (11, 12). Basic pharmacokinetics studies were not required so the optimal dose/regimen for each of the potential clinical applications of β-carotene is not known.

Some kinetic information is known. A single dose of β-carotene results in an increase in serum β-carotene that peaks 6–50 h after oral ingestion and is cleared over a period of 4–12 d (13, 14). The amount of β-carotene absorbed is a small fraction of the ingested dose and is highly variable from individual to individual. Several early pharmacokinetic studies of daily low doses (15–60 mg β-carotene/d) indicate that at these low doses a steady-state serum concentration is reached over a period of 5–20 d (15–21). A study at an intermediate dose, 180 mg/d, does not show faster serum carotene accumulation than the low-dose studies but the blood was drawn at 2-wk intervals so the kinetics were not clearly defined (22, 23). Many of these studies monitored total serum carotenoids rather than specifically measuring β-carotene, making it difficult to know exactly how the β-carotene concentrations changed.

Although several investigators reported increased β-carotene absorption in subjects on high-fat diets, it is not known whether dietary fat is essential for β-carotene absorption. The extent to which β-carotene absorption can be enhanced by administering it in association with the entire day’s fat consumption is also unknown. There is no information about the rate of accumulation of β-carotene in tissue, which may significantly lag serum accumulation. The data on accumulation of other carotenoids, some of which have been hypothesized to have greater therapeutic potential than β-carotene, are scarce (24, 25). Thus, it is difficult to know what dose, regimen, and carotenoid to administer if the goal is to deliver the maximum amount of carotenoid to serum and tissue in the shortest period of time.

Our interest relates to preferential accumulation of β-carotene in lipid-rich atherosclerotic plaques. Dietary carotenoids confer a yellow color to plaque, making it easier to identify at surgery and permitting selective removal of atherosclerotic plaque with laser radiation tuned to a carotenoid absorption peak. For this clinical application it is necessary to know how long it takes to achieve the highest tissue carotenoid concentrations. It is impractical, however, to serially sample atherosclerotic plaque in humans, so instead we monitored accumulation of β-carotene in serum and in a more accessible lipophilic tissue, skin. The goal of this study was to determine J the importance of dietary

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fat for β-carotene absorption, 2) how the rate and magnitude of β-carotene absorption are affected by spreading the dose over the entire day's fat consumption, 3) the rate and magnitude of β-carotene absorption on the maximum Food and Drug Administration-recommended dose of 300 mg/d, and 4) the rate of accumulation in the stratum corneum, a lipophilic tissue. In this study multiple carotene regimens were given to the same set of individuals to eliminate the confounding effects of human-to-human variability.

**Subjects and methods**

**Subjects**

Five healthy volunteers, three males (subjects 1, 2, and 3) and two females (subjects 4 and 5), took regimens of β-carotene under informed-consent. The study protocol was approved by the Massachusetts General Hospital Committee on Human Studies. Their characteristics are shown in Table 1. Throughout the experiments the subjects maintained their normal dietary pattern with a consistent amount of dietary fat.

**Beta-carotene**

Experiments were performed with β-carotene capsules (Cardiospectrum, Walpole, MA) containing 17 mg β-carotene, 180 mg soybean oil, 6 mg lecithin, and 35 mg wax packaged in gelatin capsules. This preparation is commonly sold in grocery, pharmacy, and health-food stores under various labels as 25 000 international units of vitamin A. The soybean oil and lecithin inhibit oxidative decomposition. The β-carotene content of a random sampling of capsules was measured periodically during the study by extracting β-carotene from the capsules, dissolving it in hexanes, and measuring the absorption spectrum of a dilute solution. The β-carotene content and purity of this single lot of capsules was stable over the course of the study with 97% trans-β-carotene and 3% 15-cis-β-carotene.

**Single-dose experiments**

To evaluate the importance of dietary fat for carotenoid absorption, subjects 2–4 took 51 mg β-carotene (3 capsules) with and without 200 g fat. In the no-fat regimen, subjects fasted for a minimum of 12 h before the study. Baseline serum samples were drawn before and during fasting. Beta-carotene (51 mg) was then administered orally followed by an additional 6 h with no dietary fat. Additional serum samples were drawn at 3, 6, 9, 12, 18, 24, 48, 72, and 168 h post-β-carotene administration.

In the regimen with fat β-carotene (51 mg) was administered with two-thirds pint ice cream containing 200 g fat (vanilla swiss almond, Haagen-Daz Co Inc, Teaneck, NJ) and serum samples were drawn at 1, 2, 3, 4, 6, 9, 12, 18, 24, 36, 48, 72, 168, 320, and 640 h post-β-carotene. All serum samples were stored at −70°C for later analysis.

**Multiple-dose experiments**

Once the importance of dietary fat for β-carotene absorption was established, a series of multiple-dose regimens was undertaken to determine how absorption and kinetics are affected by spreading β-carotene over an entire day's dietary fat and by increasing the dose to the maximum recommended (300 mg/d). The regimens were as follows: 1) 51 mg once a day with breakfast (subjects 2–5), 2) 17 mg three times a day with meals (subjects 1–5), 3) 34 mg three times a day with meals (subjects 1–4), and 4) 102 mg three times a day with meals (subjects 1–5). [Data from this regimen have been reported previously in a short communication (26).]

The order in which the regimens were undertaken was varied for each subject and there was a period of ≥ 2 mo without β-carotene supplementation between each regimen. Fasting blood was drawn and the serum stored at −70°C on day 0 (before starting β-carotene) and then approximately every Monday, Wednesday, and Friday for 3–4 wk with the exact dates dependent on the subject’s schedule. Compliance was monitored by asking subjects at the time of each blood collection if any doses had been missed. The time of onset of known side effects, including orange stool and yellow skin color, was noted. Although subjects' diets were not controlled, the importance of maintaining a consistent dietary pattern was stressed each time serum was collected and each subject’s average dietary fat content was estimated from a dietary record.

**Analysis of serum**

The frozen serum was allowed to thaw at room temperature and 0.5 mL serum was mixed with a solution of 1 μg retinyl acetate (an internal standard) in 0.5 mL ethanol. After vortexing for 20 s the ethanol-serum mixture was extracted three times with hexanes. The extracted lipids were further separated by a high-pressure liquid chromatography (HPLC) method described previously (27). This consisted of a C-18, 5-μm, reverse-phase

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**TABLE 1**

Characteristics of subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age</th>
<th>LDL y g/L</th>
<th>HDL m g/L</th>
<th>Trig 0.78</th>
<th>Chol 1.86</th>
<th>Height 1.76</th>
<th>Weight 70</th>
<th>B g</th>
<th>L g</th>
<th>D g</th>
<th>Total g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>36</td>
<td>1.75</td>
<td>0.60</td>
<td>0.78</td>
<td>2.51</td>
<td>1.76</td>
<td>70</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>31</td>
<td>1.27</td>
<td>0.46</td>
<td>0.65</td>
<td>1.86</td>
<td>1.63</td>
<td>57</td>
<td>1</td>
<td>12</td>
<td>59</td>
<td>72</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>30</td>
<td>1.19</td>
<td>0.47</td>
<td>1.02</td>
<td>1.86</td>
<td>1.70</td>
<td>73</td>
<td>8</td>
<td>56</td>
<td>73</td>
<td>137</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>31</td>
<td>0.77</td>
<td>0.92</td>
<td>0.56</td>
<td>1.80</td>
<td>1.60</td>
<td>48</td>
<td>5</td>
<td>19</td>
<td>49</td>
<td>73</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>31</td>
<td>0.96</td>
<td>0.58</td>
<td>0.57</td>
<td>1.66</td>
<td>1.70</td>
<td>57</td>
<td>12</td>
<td>28</td>
<td>48</td>
<td>88</td>
</tr>
</tbody>
</table>

* Trig, triglycerides; Chol, cholesterol.
† B, breakfast; L, lunch; D, dinner; Total, daily total; NA, not available.
column (LC18 Supelco, Bellefonte, PA) with an isocratic solvent (acetonitrile-methylene chloride-methanol, 70:20:10) flowing at 1.7 mL/min. For each specimen, the hexanes were evaporated under nitrogen and residual lipids resuspended in 0.5 mL of the mobile phase of the column.

The absorption of β-carotene and other carotenoids was monitored at 450 nm, whereas retinol (vitamin A) and retinyl acetate (the internal standard) were monitored at 327 nm. The absolute amounts of β-carotene, lycopene, retinol, and retinyl acetate were determined from the areas under each chromatogram peak. The HPLC was periodically calibrated with known amounts of each compound and the purity of the β-carotene, lycopene, retinol, and retinyl acetate standards was assessed by comparing the absorption spectrum of each compound with its known extinction coefficient [Em 2590, 3450, 1835, and 1510, respectively (28)]. Extraction yield was determined as the fraction of extracted retinyl acetate.

The statistical significance of changes in serum concentrations of the measured species was calculated with Student's t test by using the computer program Excel (4.0; Microsoft, Seattle, WA). On the multiple-dose regimens the values at time 0 were compared with those measured on or extrapolated to day 20 of each regimen.

Serum lipids, including high-density lipoprotein, low-density lipoprotein (LDL), triglycerides, and cholesterol, were measured by the hospital chemistry laboratory on serum taken at multiple times during the course of the study.

To determine an approximate serum-rise time constant, \( r \), the serum concentrations were fitted to a first-order model for serum β-carotene accumulation given by

\[
\text{serum } \beta\text{-carotene} = A \left(1 - \exp(-t/r)\right) + \text{baseline serum } \beta\text{-carotene} \quad (1)
\]

where \( A \) is the maximum increase in serum β-carotene and \( t \) is time. The data show, however, that the kinetics are more complex; a multicompartment model would be required to fit the data precisely.

Remittance spectroscopy

To evaluate the rate at which β-carotene accumulates in the lipophilic stratum corneum, remittance spectra of palms were obtained on three of the subjects during their final regimen (34 mg three times a day). A spectrophotometer (Beckman Instruments Inc, Fullerton, CA) fitted with a 15-cm integrating sphere that included a port to exclude specular reflectance was used to measure remittance from 600 to 300 nm in 1-nm increments. Data were obtained from a 1-cm² region of skin on the right palm (thenar eminence) for each time point. The device was also calibrated at each time point by obtaining a spectrum with the sample beam blocked (zero remittance) and a spectrum with a 100% remitting plate freshly coated with barium sulfate (white reflectance coating no. 6080, Eastman Kodak Co, Rochester, NY). Absorbance was determined by calculating −log(remittance) for each day. The day 0 scan was subtracted from later spectra to see changes in the absorption because of β-carotene. Although the absolute amount of β-carotene could not be determined from remittance spectra because the skin scattering coefficient for each individual subject was not known, it was possible to determine the relative amount of β-carotene in skin by measuring the β-carotene peak heights for each scan.

Results

Single-dose experiments

Beta-carotene administered after a period of fasting with no dietary fat showed no detectable accumulation in the serum (see Fig 1). When administered with 200 g fat, however, 2.8% of the administered β-carotene was absorbed, resulting in a 2.5-fold increase in serum β-carotene. The increase in serum β-carotene began 3–6 h after administration and peaked at 36–48 h.

Multiple-dose experiments

On each multiple-dose regimen, the serum β-carotene reached a steady-state value with a time constant of 9–10 d as shown in Fig 2, A–D. The stool turned orange at ~3–5 d and the skin began to turn yellow at ~10 d in all subjects on the regimen of 17, 34, and 102 mg three times a day but not on the regimen of 51 mg once a day. None of the subjects found these side effects bothersome and compliance in the five subjects appeared to be 100%. The observed serum β-carotene and total carotenoid concentrations on the regimen of 51 mg once a day are comparable with those reported in earlier studies on similar regimens (14, 16, 19, 29).

Comparison of the same total amount given in one dose (with breakfast) vs the amount given in three divided doses with meals (Fig 2, A and B) reveals that three times greater absorption was obtained when the β-carotene was administered over three meals \((P < 0.001)\) (see Tables 2 and 3). As the β-carotene dose increased from 17 to 34 to 102 mg three times a day with meals, the average steady-state serum β-carotene concentration rose from 3.4 to 7.1 mg/L but the time constant (10 d) was not significantly affected (Fig 2 and Table 3). Figure 3 shows the serum concentration obtained by each subject as a function of dose and the average of all subjects at day 20 on each regimen. The baseline value was lowest for 51 mg once a day for two reasons. For three of the subjects, this was their first regimen; for the other two subjects, the interval between the regimen of 51 mg once a day and the previous regimen was longer than the other intervals.

![FIG 1. Serum response to a single dose of β-carotene for a representative subject with and without dietary fat. After 51 mg β-carotene taken during fasting, there is no appreciable change in serum β-carotene. The same dose administered with 200 g fat shows a fourfold increase in β-carotene peaking at 40 h.](image-url)
On subsequent regimens the baseline never completely returned to its original concentration despite waiting ≥ 2 mo in between regimens.

At the highest doses, 34 and 102 mg β-carotene three times a day, the serum concentrations of other carotenoids began to decrease (P < 0.05). Figure 4 shows the average serum concentrations of one of the other carotenoids, lycopene, for each of the regimens of three times a day. Retinol (vitamin A) showed no significant change on any of the regimens. No significant

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**TABLE 2**
Serum carotenoid and vitamin A response to 20 d on oral β-carotene

<table>
<thead>
<tr>
<th>Dose</th>
<th>β-carotene</th>
<th>Carotenoids</th>
<th>Vitamin A</th>
<th>Lycopene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (n = 5)</td>
<td>0.33 ± 0.18</td>
<td>1.76 ± 0.36</td>
<td>0.37 ± 0.07</td>
<td>0.32 ± 0.12</td>
</tr>
<tr>
<td>51 mg once a day (n = 4)</td>
<td>1.1 ± 0.3†</td>
<td>2.21 ± 0.25</td>
<td>0.31 ± 0.03</td>
<td>0.21 ± 0.06</td>
</tr>
<tr>
<td>17 mg three times a day (n = 5)</td>
<td>3.2 ± 0.3§</td>
<td>4.67 ± 0.32§</td>
<td>0.35 ± 0.09</td>
<td>0.28 ± 0.12</td>
</tr>
<tr>
<td>34 mg three times a day (n = 4)</td>
<td>3.7 ± 1.5†</td>
<td>4.88 ± 1.60†</td>
<td>0.43 ± 0.06</td>
<td>0.26 ± 0.11†</td>
</tr>
<tr>
<td>102 mg three times a day (n = 5)</td>
<td>6.1 ± 1.0§</td>
<td>7.39 ± 0.91§</td>
<td>0.39 ± 0.05</td>
<td>0.25 ± 0.13†</td>
</tr>
</tbody>
</table>

* σ ± SD. Baseline calculated from values unaffected by a prior β-carotene regimen.
† †§ Significantly different from baseline: P < 0.05; † †§ P < 0.01, † † †§ P < 0.001.

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**TABLE 3**
Beta-carotene serum kinetics for each regimen

<table>
<thead>
<tr>
<th>Dose</th>
<th>Baseline</th>
<th>Steady-state</th>
<th>Rise time</th>
<th>Decay time</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg</td>
<td>mg/L</td>
<td>mg/L</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>51 mg once a day (n = 4)</td>
<td>0.36</td>
<td>1.14</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>17 mg three times a day (n = 5)</td>
<td>0.49</td>
<td>3.42</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>34 mg three times a day (n = 4)</td>
<td>0.53</td>
<td>4.12</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>102 mg three times a day (n = 5)</td>
<td>0.68</td>
<td>7.06</td>
<td>10</td>
<td>15</td>
</tr>
</tbody>
</table>

* Baseline based on serum β-carotene concentrations unaffected by a prior regimen is 0.33 mg/L for all subjects.
correlation between any of the measured lipids and the serum \(\beta\)-carotene or total carotenoid concentration was identified, although \(\beta\)-carotene is known to be transported in serum by LDLs (30).

When oral \(\beta\)-carotene was stopped, serum \(\beta\)-carotene decayed approximately exponentially (see Fig 5) with a half-life of 10 d that did not vary appreciably with dose. Because the small bowel continued to release \(\beta\)-carotene into the bloodstream for several days after the final oral dose, the serum decay cannot be adequately modeled with a single exponential. The stool returned to normal within a few days and the skin color returned to normal within 2 mo of discontinuing \(\beta\)-carotene.

**Tissue accumulation of \(\beta\)-carotene**

The characteristic three-peaked \(\beta\)-carotene absorption profile is clearly evident in absorption spectra derived from skin remittance data (see Fig 6). The observed \(\beta\)-carotene absorption maxima in skin at 475, 490, and 510 nm are red-shifted \(\approx\) 40 nm relative to those maxima in hexanes. Carotenoid absorption spectra are typically red-shifted as the polarity of the solvent decreases and stabilizes the excited electronic states. There may...
also be some red shift related to the wavelength-dependent differential penetration depth of visible light in skin. The absorption feature at 370 nm (Fig 6) was observed consistently in some but not in all subjects. It could be due to cis-β-carotene or possibly a carotenoid metabolite or an impurity.

The relative amount of β-carotene in skin was calculated from the change in absorption at 490 nm and is plotted as a function of time in Figure 7. In subject 4, the absorbance at 490 nm closely followed serum β-carotene concentrations with no delay; in subjects 2 and 3 the increase in skin absorption was delayed by ≈ 2 wk relative to the serum accumulation. This delay may reflect differences in skin thickness that can vary considerably from one individual to the next. Other possible explanations would include variable rates of uptake into the basal cells and subcutaneous fat.

**Discussion**

Beta-carotene has therapeutic promise but the optimal dose and regimen for each of its potential applications has been difficult to predict. These data on serum and skin β-carotene accumulation demonstrate important aspects of β-carotene absorption and tissue accumulation that should influence its therapeutic application and potential.

Beta-carotene absorption into the body is known to increase when it is taken in association with a high-fat diet. These data confirm that observation and further reveal that essentially no β-carotene is absorbed in the absence of dietary fat. The increase in serum β-carotene achieved by dividing the β-carotene dose over three meals as compared with a once-a-day regimen enables significant (threefold) enhancement of β-carotene absorption without having to increase dietary fat. This regimen is counterintuitive because pharmaceuticals with a half-life of weeks are not generally administered three times a day. The clinical trials of β-carotene to prevent cancer and atherosclerosis using 50 mg taken every other day might be improved by administering the same total amount of β-carotene in divided doses with meals. Another possibility is to administer a once-a-day regimen with the daily meal that contains the most fat. Other previously reported factors associated with increased β-carotene absorption include being a nonsmoker, female, or lean (31) but their impact appears to be far less than that of the effect of dietary fat observed in this study.

These data demonstrate that a multicompartment model is required to completely analyze β-carotene absorption. The small bowel accepts a bolus of lipid within a few hours of eating but releases the β-carotene into the serum over a much longer period, exceeding 50 h. This presumably reflects the time required for the β-carotene to be absorbed into intestinal mucosal cells, packaged with lipids, and then released as chylomicrons. With continuous oral administration the serum is observed in this study to accumulate β-carotene with a time constant of the order of 9–10 d. The remittance data indicate that skin accumulation may be delayed by up to 2 wk compared with serum accumulation. This time scale of tissue accumulation is consistent with data reported for β-carotene accumulation in buccal mucosa (29). Accumulation in other tissues may be different. Thus, there are at least three compartments with different time constants for β-carotene accumulation and perhaps more.

Because it is unlikely that any tissue other than the gastrointestinal tract could accumulate β-carotene faster than it accumulates in serum, the potential therapeutic applications of β-carotene, including treating erythropoietic protoporphyria (12), tumor therapy (5), or enhancing laser angioplasty (9), which demand the highest possible carotene concentrations, are likely to require several weeks on oral β-carotene before the maximum therapeutic benefit can be expected. Serum accumulation did not increase linearly with increasing dose (Fig 3) and at the highest dose administered (100 mg three times a day) other carotenoids were decreasing as β-carotene increased. These findings suggest that at high doses β-carotene competes with other carotenoids for absorption. If this is the case, then only a minimal additional increase in total serum carotenoids can be achieved by increasing the β-carotene dose further. Higher and more rapid elevation of serum β-carotene may require intravenous administration.

The spectral shift of β-carotene absorption in tissue as compared with hexanes and other solvents is particularly important to consider in using β-carotene to enhance laser angioplasty because the laser chosen must emit at the carotene absorption peak. If the β-carotene absorption shift in atherosclerotic plaque is comparable with that observed in skin, then the optimal wavelength for selective laser angioplasty post-β-carotene loading would be in the 470–510-nm range. Fortunately, several lasers emit in this spectral region, including the flashlamp-excited dye laser, doubled titanium sapphire (a solid-state laser), argon ion (a gas laser), selenium (a semiconductor laser), and the argon fluoride excimer laser (C → A transition).

The side effects observed all have been reported previously. The yellow skin color may be a useful marker for evaluating compliance and determining when and which lipophilic tissues have accumulated significant amounts of pigment. These results also agree with previous studies that show that vitamin A toxicity does not result from high provitamin A (β-carotene) concentrations, presumably because of feedback inhibition.

We thank Margo Goetschkes, Glenn LaMuraglia, Jerry Stringham, Roxanne Sylora, Micheline Mathews-Roth, Norm Krinsky, Adrienne Bendich, Irene Kochevar, Tayabba Hasan, and John Parrish for many helpful suggestions.

![Figure 7](image-url) Change in 490-nm absorbance in skin over the thenar eminence with time on β-carotene (34 mg three times a day). The accumulation of β-carotene in the skin of subjects 2 and 3 is delayed by 2 wk compared with its accumulation in their serum.
References