Stability of Serum Carotene at Various Light and Temperature Conditions

To the Editor.—Carotenoids are brightly colored plant pigments that are essential for several metabolic functions including cell growth regulation and immune response. Carotene (pre-vitamin A) is a slowly absorbed, fat-soluble, carotenoid pigment normally present in the diet. Serum carotene levels are used to determine the possibility of absorption defects that can lead to malabsorption. Decreased carotene levels are seen in several malabsorption syndromes, including, but not limited to, some pancreatic diseases, sprue, post–small intestine resection, and celiac disease.

Fat-soluble vitamins, such as carotene, are generally considered highly unstable when exposed to light, air, and ambient temperature. Therefore, serum carotene samples that are referred to core laboratories from satellite sites are required to be shipped frozen and protected from light at all times of handling and analysis. Recent studies indicate that fat-soluble vitamins, including carotene concentrations in whole blood, change insignificantly when stored for up to 7 days at room temperature in dark or light conditions; however, stability data for serum carotene remain scarce.

We prepared 5 different serum pools from healthy adult volunteers and from 7-day-old leftover laboratory specimens within our laboratory. Aliquots of 600 μL from each pool were maintained at various light and temperature conditions: room temperature–dark, room temperature–light, refrigerated-dark, refrigerated-light, frozen (−30°C)-dark, and frozen (−70°C)-dark for durations of 1 to 14 days. At the end of the timed conditions (Day 0, 1, 3, 7, 10, 14), the appropriate aliquots were removed and stored at −70°C until analysis. Serum carotene was then extracted with petroleum ether and analyzed using spectrophotometry at a wavelength of 440 nm. The procedure was performed under minimal lighting conditions. The stability of carotene under each storage condition was determined by calculating the percentage change in concentration from those of day 0, then calculating the mean percentage change (and standard error of the mean) of the 5 pools at each time point. Data were analyzed by 1-way analysis of variance using SPSS (Chicago, Illinois) and a P value < .05 was considered statistically significant.

The analytic imprecision of this carotene assay is 6.6% at levels of 96.6 μg/dL (1.8 μmol/L) and 155.7 μg/dL (2.9 μmol/L). Carotene levels of the 5 serum pools ranged from 134.2 μg/dL (2.50 μmol/L) to 230.9 μg/dL (4.3 μmol/L). Serum stored at −70°C for 14 days (the end of study controls) demonstrated a minimal average change of only 3.04% compared with those measured freshly at day 0. Samples stored in the room temperature–light condition for 10 and 14 days demonstrated a significant decrease of 24.5% (P = .02) and 28.3% (P = .01) respectively in carotene levels when compared with the end-of-study controls. Except for room temperature, storage conditions did not induce clinically significant changes in serum carotene (Table).

In conclusion, serum carotene levels are stable when stored at 4°C (in dark or light) or in the freezer for up to 14 days. Carotene stability is also acceptable at room temperature–dark conditions.

### Percentage Change of Serum Carotene (SE) at Various Conditions

<table>
<thead>
<tr>
<th>Day</th>
<th>Room Temperature</th>
<th>Refrigerated (4°C)</th>
<th>Frozen (−30°C)</th>
<th>Frozen (−70°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dark</td>
<td>Light</td>
<td>Dark</td>
<td>Light</td>
</tr>
<tr>
<td>1</td>
<td>2.05 (3.25)</td>
<td>−3.77 (2.31)</td>
<td>−0.60 (4.51)</td>
<td>−6.23 (3.74)</td>
</tr>
<tr>
<td>3</td>
<td>−1.54 (2.85)</td>
<td>−5.95 (2.77)</td>
<td>−2.97 (4.08)</td>
<td>−2.15 (4.71)</td>
</tr>
<tr>
<td>7</td>
<td>−6.90 (3.94)</td>
<td>−14.52 (2.77)</td>
<td>−2.40 (4.49)</td>
<td>−8.35 (4.31)</td>
</tr>
<tr>
<td>10</td>
<td>−11.91 (3.34)</td>
<td>−24.45 (4.18)a</td>
<td>−3.24 (3.66)</td>
<td>−9.87 (4.18)</td>
</tr>
<tr>
<td>14</td>
<td>−14.60 (4.75)</td>
<td>−28.25 (3.88)b</td>
<td>−3.62 (3.56)</td>
<td>−9.64 (4.07)</td>
</tr>
</tbody>
</table>

Abbreviation: SE, standard error of mean.

* P = .02 compared with percentage change in serum carotene frozen at −70°C for 14 days.

* P = .01 compared with percentage change in serum carotene frozen at −70°C for 14 days.

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for 7 days or room temperature–light for 3 days. In addition, repeated freezing to $-70^\circ C$ and thawing has been demonstrated to have no significant effects (less than 1% per freeze-thaw cycle) on the serum and plasma carotene concentrations. These results allow for less stringent storage, shipping, and handling conditions for serum carotene specimens.

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