Concurrent Liquid-Chromatographic Assay of Retinol, α-Tocopherol, β-Carotene, α-Carotene, Lycopene, and β-Cryptoxanthin in Plasma, with Tocopherol Acetate as Internal Standard

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A method is described for simultaneously determining retinol, α-tocopherol, β-carotene, α-carotene, lycopene, and β-cryptoxanthin in 0.25 ml of plasma. Plasma mixed with sodium dodecyl sulfate is deproteinized with ethanol containing tocopherol acetate, then extracted with heptane. The evaporated protein film is reconstituted with mobile phase (methanol:acetone/trichloroform, 47/47/6 by vol) and injected onto a 100 × 4.6 mm 3-μm column of Spherisorb ODS-2 (LKB) at 1.5 ml/min. The α- and β-carotenoids are well resolved during the 6.5-min run. Retinol is monitored at 325 nm, the tocopherols at 292 nm, and the carotenoids at 450 nm. Extraction of concentrations as great as 135 μmol/L is complete. Interbatch CVs were 1.7%, 2.3%, 4.1%, 10.4%, 6.4%, and 3.6% for retinol, α-tocopherol, β-carotene, α-carotene, lycopene, and β-cryptoxanthin, respectively. Interbatch CVs for measurements on 30 occasions over 11 weeks were about 10% for all components except α-tocopherol (5.3%). Results agree well with those for retinol, α-tocopherol, and β-carotene in quality-control samples.

Additional Keyphrases: factors affecting sensitivity • cancer screening • nutritional status

It has been generally assumed for many years that β-carotene was a major component of plasma carotenoids, about 50% being considered a useful approximation (1). It is therefore not surprising that, after recent suggestions inversely relating the incidence of certain cancers to the intake of dietary carotenoids or to plasma carotene concentrations (2), several studies have appeared that examined and generally supported (3–5) the relationship between plasma β-carotene and cancer incidence. However, β-carotene does not always predominate in blood (6); one recent study illustrated this for a group of Canadians in whom β-carotene was only 14% of the total carotenoids (7), a result that we have also confirmed for subjects in the U.K. (8). Thus a disproportionate amount of attention perhaps is being given to β-carotene; for although it is an anti-oxidant and capable of working at low oxygen pressures (9), the structure of all carotenoids is very similar and most quench singlet oxygen very effectively (10). It may therefore be important to monitor more than just β-carotene.

Thompson et al. (7) have reported as many as 20 regularly occurring carotenoids in normal serum, but there are only three main pro-vitamin A carotenoids in blood: α-carotene, β-carotene, and β-cryptoxanthin (3-hydroxy-β-carotene) (11); lycopene, a straight-chain isomer of β-carotene, also is usually present in substantial amounts (1, 12). Lycopene has no vitamin A activity, but tomatoes, in which the carotene is primarily lycopene, were recently associated with a protective action against cancer (13).

The current interest in the possible protective role exerted by retinoids and anti-oxidants against cancer (14) has stimulated the introduction of several methods for the combined analysis of retinol, α-tocopherol, and the carotenoids in serum (12, 15–17). Perhaps the most useful of these for screening purposes is the method of Milne and Botnen (12), which is relatively quick (9 min) and can be used with samples as small as 200 μL. Here, we describe an alternative method with equivalent or higher sensitivity, reliability, and a usual run time of only 6.5 min. We specifically measure the three pro-vitamin A carotenoids plus lycopene, retinol, and α-tocopherol and we are using the method in an ongoing nutrition survey of 2000 adults in the U.K. (8).

Materials and Methods

Materials. All-trans-β-carotene, all-trans-α-carotene, lycopene, α-tocopherol, tocopherol acetate, and retinol, all in pure crystalline form, were obtained from Sigma Chemical Co., Poole, Dorset, U.K. β-Cryptoxanthin was a gift from Hoffmann-La Roche Co. Ltd., Basel, Switzerland. HPLC-grade acetonitrile, n-hexane, n-heptane, and chloroform were obtained from Rathburn Chemicals Ltd., Walkernburn, Peebleshire, U.K.4 Methanol of "HPLC" quality or better was obtained from various sources in the U.K. (see below). Absolute ethanol (Spectrosol), and sodium dodecyl sulfate (SDS) were obtained from British Drug Houses, Poole, Dorset, U.K. Butylated hydroxytoluene (BHT) was obtained from Koch-Light Ltd., Haverhill, Suffolk, U.K.

Chromatography. The HPLC system comprised a Waters 501 pump, a Wisp 710B autosampler, a four-channel 490 detector (all from Millipore Waters, Harrow, Middlesex, U.K.) and three integrator/chart recorders. The mobile phase (acetonitrile/methanol/chloroform, 47/47/6 by vol) was filtered (2-μm pore-size PTFE filters; Whatman Ltd., Maidstone, Kent, U.K.), degassed by sonication for 30 min, and pumped at 1.5 mL/min. We separated 25-μL extracts in mobile phase isocratically on a 100 × 4.6-mm cartridge column of 3-μm particles of Spherisorb ODS-2 (LKB Instruments Ltd., South Croydon, Surrey, U.K.). We did not use the cartridge guard column provided with this equipment because of problems associated with the column connector. To avoid blockage of the column, we placed a 0.5-μm pore-size stainless steel frit (Anachem Ltd., Luton, Bedfordshire, U.K.) just ahead of the column and changed it daily. Two channels were used for detection. Channel 1 was linked to a CI-10B integrator (LDC Ltd., Stone, Staffs., U.K.). It was programmed to monitor at 325 nm from 0 to

4 Nonstandard abbreviations: BHT, butylated hydroxytoluene; HPLC, "high-performance" liquid chromatography; SDS, sodium dodecyl sulfate; PTFE, polytetrafluoroethylene.
1.5 min and at 292 nm from 1.5 to 3.5 min, with 1.0 A full-scale throughput. A second channel was linked to two Model 740 integrators (Millipore Waters Ltd.) and programmed to monitor at 450 nm and 0.05 A full-scale throughput. One recorder monitored the entire output as area percent, the other monitored the concentrations of the four specific peaks.

Sample preparation. Mix briefly 0.25 mL of plasma or serum with 0.25 mL of 10 mmol/L SDS reagent (18), continuing for 1 min after adding 0.5 mL of ethanol containing 40 μmol of tocopherol acetate per liter. Finally add 1 mL of 0.7% SDS reagent containing 0.5 g of BHT per liter, vortex-mix vigorously for 2.5 min, and centrifuge (2500 × g, 10 min, 20 °C) to separate the phases. Remove 0.7 mL of the supernatant heptane, evaporate under nitrogen at 40 °C, and reconstitute the residue in 0.25 mL of mobile phase. For small samples, mix 0.1 mL with 0.1 mL of SDS, 0.2 mL of ethanol, and 1 mL of heptane. Remove 0.7 mL of heptane, evaporate, and reconstitute in 0.1 mL of mobile phase for measurement as above.

Protection of samples and standards from light. We protected samples by extracting under dimmed natural lighting, excluding direct sun and fluorescent light at all times. The final extracts were placed in amber-colored vials containing 0.3-mL disposable polyethylene inserts (Hughes & Hughes Ltd., Romford, U.K.).

Stock standards were stored at −20 °C; we prepared working standards under natural lighting as above. Individual and mixed working standards were protected from light at all times and kept at 4 °C when not in use.

Quantification. We prepared individual stock standards as shown in Table 1 and stored them at −20 °C. Response factors were calculated at the start of each week, or when new batches of reagents were used, or after any adjustment to the equipment. Working standards were prepared in appropriate solvents and their concentrations calculated from their absorbivities (Table 1) and adjusted for purity. Purity was assessed from the second set of working standards prepared in mobile phase; these were chromatographed individually and the area of each standard was expressed as a proportion of the total area recorded. Lycopene was typically only 30% pure, so we prepared new stock solutions of it every seven to 14 days; all the other stock standards were sufficiently stable to be used for six to eight weeks or longer. We prepared a working internal standard for the extraction procedure weekly from the stock (0.2 mL/100 mL ethanol).

The sensitivity of the system was checked daily with a mixed working standard (Table 1) and the peak areas were recorded. Likewise, the peak area recorded for the working internal-standard solution (0.5 mL of solution evaporated and reconstituted in 0.25 mL of mobile phase) was checked daily to confirm that it was within the previously established range (± 2SD).

Recovery experiment. Analytical recovery was measured by a slightly modified form of the above method. Ethanol (0.5 mL) containing internal standard (approx. 40 μmol/L) was pipetted into 18 extraction tubes (groups A, B, and C), six of which (group A) contained 0.25 mL of mixed working standard that had been previously evaporated to dryness. After mixing 5.0 mL of serum with 5.0 mL of 10 mmol/L SDS reagent, we added 0.5 mL of the serum/SDS mixture to each of the 18 tubes, mixed, added 1.0 mL of heptane/BHT, vortex-mixed vigorously for 2.5 min, and carefully removed 0.7 mL of the heptane extract from each tube. We then evaporated these portions of the extracts and reconstituted them as follows: Group A, with 0.25 mL of mobile phase; Group B, with 0.25 mL of mobile phase; and Group C, with 0.25 mL of mixed working standard in mobile phase.

We then made single measurements of retinol, tocopherol, and tocopherol acetate in each of the 18 extracts by using the external standard method of calibration (Table 2).

Asay precision. To determine the interbatch coefficient of variation (CV), we assayed daily 0.5-mL portions of stored plasma from a date-expired, blood-transfusion sample stored in 5-mL plastic tubes at −20 °C. No sample was frozen and thawed more than twice before use. The intra-batch CV was obtained from single measurements of extracts from 20 aliquots of the same plasma sample.

Results and Discussion

Vitamin extraction. The inclusion of SDS into the method is not essential for the extraction of fat-soluble vitamins, and most workers mix equal volumes of sample and alcohol (12, 20, 21). The use of SDS was introduced by Burton et al. (19), who were interested in measuring vitamin E. SDS enables an efficient extraction of lipid from tissue or serum into the heptane so a single extract can be used for measurements of vitamin E:lipid ratios, which is needed to assess vitamin E status (22, 23). We adopted this procedure because our initial interests involved tocopherol; however, the modification has proved to have no disadvantages in the measurement of retinol and carotenoids.

Table 1. Calibrating the Assay

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Absorptivity, A·mol⁻¹·cm⁻¹ (at λ, nm)</th>
<th>Stock standard concn (and solvent)*</th>
<th>For calibration</th>
<th>To adjust for purity b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol</td>
<td>52.48 (328)</td>
<td>25 mg/100 mL (ethanol)</td>
<td>1:50 (ethanol)</td>
<td>1:500</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>3.26 (252)</td>
<td>1 g/100 mL (heptane)</td>
<td>1:100 (ethanol)c</td>
<td>1:1000</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>145.5 (446)</td>
<td>1 mg/10 mL (hexane)</td>
<td>1:100 (hexane)</td>
<td>1:500</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>136.91 (452)</td>
<td>5 mg/25 mL (hexane)</td>
<td>1:100 (hexane)</td>
<td>1:500</td>
</tr>
<tr>
<td>Lycopene</td>
<td>186.3 (474)</td>
<td>1 mg/10 mL (chloroform)</td>
<td>1:11 (hexane)c</td>
<td>10:50</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>136.0 (451)</td>
<td>0.5 mg/10 mL (hexane)</td>
<td>1:20 (hexane)</td>
<td>2:500</td>
</tr>
<tr>
<td>Tocopherol acetate</td>
<td>(284)</td>
<td>1 g/100 mL (heptane)</td>
<td>1:100 (ethanol)c</td>
<td>2:500</td>
</tr>
</tbody>
</table>

*a Hexane and heptane solvents contain 500 mg of BHT per liter.

*b Stock solvent was evaporated and residues were diluted in mobile phase. Working standards were used individually to assess purity and to correct the concentrations of working standards before calculating the response factors, or as a mixed working standard to monitor sensitivity daily.

*c Stock solvent was removed by evaporation under nitrogen before dilution with solvent listed in parentheses.

*d Only to check stability.

*Added in the preparation of the mixed working standard only.
The results in Table 2 show that 70% of the retinol, α-tocopherol, and tocopherol acetate added to the tubes in group A was recovered in the final extract. In effect, the recovery was 100% because only 0.7 mL of the 1.0 mL of heptane added to each tube was used for the final measurements. The concentrations measured in this experiment ranged from 1.45 to 135 μmol/L; thus, recovery is complete for concentrations within this range. Although the amount of tocopherol acetate was very much greater than that of retinol, the efficiency of extraction was the same.

Although it has been suggested that this type of recovery experiment can be misleading because lipoprotein-bound substances in plasma are less readily extracted than those added in solvents (18), other information supports our belief that recovery is complete. For example, the results in Table 3 show very similar values for retinol, tocopherol, and β-carotene obtained by us and by the U.S. National Bureau of Standards, using different methods.

Internal standard. Our initial extractions were done with retinyl acetate, which had been used successfully by Milne and Botnen (12), as internal standard. However, in our system, retinol does not separate well from the acetate, and intra-batch precision was poor. On the other hand, we usually achieve baseline separation between tocopherol and tocopherol acetate. A relatively large concentration of tocopherol acetate has to be used to produce a peak about the same size as that for tocopherol—in part because tocopherol acetate cannot be measured at its maximum absorbance (284 nm), there being insufficient time to program a wavelength change between the times when the peaks are eluted.

We also examined C_{45}-β-carotene (24) for use as an internal standard (gift from Dr. F. Khachik, U.S. Department of Agriculture, Beltsville, MD). Its retention time was longer than that of β-carotene and well-separated from the latter, but unfortunately we found that the substance was contaminated with most of the carotenoids of interest, and attempts to purify it have so far been unsuccessful.

**Precision.** Table 4 shows the results obtained for inter- and intrabatch precision. The poorest results for intra-batch precision are obtained for lycopene and α-carotene. There are at least two isomers of lycopene in serum extracts; these are only partly separated (Figure 1) and usually are integrated together. Variations in this peak may thus be due to instability and changes in proportions of the isomers, lycopene being the least stable of our standard solutions. However, α-carotene is usually well separated from β-carotene and any preceding material but it is the least of these components measured in all U.K. serum samples.

Our results are slightly better than those reported by others (21), who obtained CVs of 13.5%, 11.8%, and 6.3% for lycopene, α-, and β-carotene, respectively. In addition, the sensitivity of the assay would appear to be better than that of Milne and Botnen (12), who failed to detect α-carotene in four of 12 samples. We have rarely failed to measure α-carotene in >1000 normal samples measured so far (8).

Interbatch precision was poorer than intrabatch precision. Several factors may contribute to this: instability of the plasma components, changes in sensitivity and coloration factors, alterations in column performance, etc. The results for interbatch precision were collected over the first three months of 1987, during which >700 samples were analyzed with the same column. However, the same plasma had also been used over the previous three months, and essentially the same values were obtained at the outset as the means shown in Table 4; that is, there has been little or no deterioration in the plasma components over six months.

The higher interbatch CV for retinol may be due to

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**Table 2. Analytical Recovery of Retinol, α-Tocopherol, and Tocopherol Acetate**

<table>
<thead>
<tr>
<th></th>
<th>Retinol, μmol/L</th>
<th>α-Tocopherol, μmol/L</th>
<th>Tocopherol acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>A: serum + working standard</td>
<td>2.81</td>
<td>0.18</td>
<td>37.14</td>
</tr>
<tr>
<td>B: serum only</td>
<td>1.45</td>
<td>0.08</td>
<td>17.52</td>
</tr>
<tr>
<td>C: serum reconstituted with working standard</td>
<td>3.39</td>
<td>0.13</td>
<td>44.75</td>
</tr>
<tr>
<td>% recovery</td>
<td>70.10</td>
<td>72.05</td>
<td>69.07</td>
</tr>
</tbody>
</table>

*a = six extractions each, as described in Materials and Methods, except that we added sample/SDS mixture to ethanol, and for group A incorporated the mixed-working standard of retinol, α-tocopherol, and tocopherol acetate into the ethanol.

*b: To convert to mg/L, multiply retinol × 0.292, α-tocopherol × 0.4307, and carotenoids × 0.5369.

*c: Calculated as [(A–B) / (C–B)] × 100.

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**Table 3. Results for Three Lyophilized Quality Control Sera**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Sample l.d. no.</th>
<th>Our results *</th>
<th>NBS *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol, μmol/L</td>
<td>50</td>
<td>1.39 (0.052)</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>1.69 (0.031)</td>
<td>1.54</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>3.33 (0.041)</td>
<td>3.26</td>
</tr>
<tr>
<td>β-Carotene, μmol/L</td>
<td>50</td>
<td>0.127 (0.001)</td>
<td>0.123</td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>1.745 (0.020)</td>
<td>1.934</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>0.935 (0.047)</td>
<td>1.038</td>
</tr>
<tr>
<td>α-Tocopherol, μmol/L</td>
<td>50</td>
<td>21.80 (0.485)</td>
<td>20.15</td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>18.85 (0.440)</td>
<td>16.61</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>29.04 (0.690)</td>
<td>24.69</td>
</tr>
</tbody>
</table>

*a: The samples, prepared by the National Bureau of Standards (NBS), originated from heparinized plasma that was blended, fortified with analyte, and lyophilized for distribution.

*b: Mean (and SD) of three determinations.

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**Table 4. Intra- and Interbatch Precision**

<table>
<thead>
<tr>
<th>Nutrient, μmol/L</th>
<th>intrabatch (n = 20)</th>
<th>Interbatch (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Retinol</td>
<td>1.265</td>
<td>0.021</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>15.404</td>
<td>0.346</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>0.278</td>
<td>0.010</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.532</td>
<td>0.034</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>0.067</td>
<td>0.007</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>0.369</td>
<td>0.015</td>
</tr>
</tbody>
</table>

*a: Different serum samples were used for inter- and intrabatch measurements. Serum for interbatch precision determinations was stored at −20°C and fresh portions were used for each assay over an 11-week period.

*b: One measurement of retinol was excluded because it was more than 3 SD above the mean.

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Changes in the sample with age. We think it is more probable, however, that the small peak immediately preceding retinol (Figure 1A), which reflects BHT and background absorbance from carotenoids, may interfere with the measurement of retinol. Although it is identified as a separate peak, variations in the way the sample is integrated and the baseline is drawn will contribute to the higher interbatch CV. However, the results are within the degree of variability expected for this type of analysis (21) and although, with the exception of tocopherol, the interbatch CVs are higher than those reported by other workers (cf. results in Table 4 with 3.1%, 6.9%, 6.1%, and 6.5% for retinol, tocopherol, lycopene, and β-carotene, respectively (12)), the latter study covered only five days.

Sensitivity. Our early experience showed us that changes in the sensitivity of the assay, particularly for detecting the carotenoids, were associated with two main factors: the column and the use of methanol. The 3-μm-particle LKB column was designed to consist of a 3-cm-long integral guard column linked by a PTFE connector to a 6-cm-long main column. These combined columns had to be replaced fairly frequently, owing to blockages, and we found variations in sensitivity with different columns. On one occasion we found that sensitivity was doubled for the carotenoids when the guard column and connector were omitted, leaving only a single 10-cm-long column. The incorporation of a 0.5-μm in-line, stainless steel frit lengthens the life of the column and its use is not associated with any alteration in sensitivity. We currently change frits daily, cleaning them by sonication in methanol; in this way, we have used a 10-cm column to separate >700 samples.

However, the main cause of variations in sensitivity in detecting carotenoids was associated with different batches of HPLC-grade methanol. A fivefold difference in sensitivity is possible, but we have not yet been able to identify the cause. We calculate new response factors regularly, allowing a ±10% variation for the carotenoids. Despite these adjustments, changes in sensitivity undoubtedly contribute to the fact that interbatch CVs are greater than intrabatch CVs. Response factors obtained for the different carotenoids are all approximately the same, although that for β-carotene is usually a little higher (10%) than the others. Whatever factors were affecting sensitivity had a similar effect on all the carotenoid response factors but their effect on tocopherol and retinol was much smaller.

Accuracy of measurements. The accuracy of this method in measuring the fat-soluble nutrients is dependent on three main factors: (a) correct calculation of standard concentrations, (b) the same extraction behavior for the internal standard and the nutrients being measured, and (c) consistent resolution, particularly of the carotenoids. We use absorbivities to calculate standard concentrations, but, in our experience, it is particularly important with regard to all the carotenoids to take purity into account as well.

Table 3 shows data obtained from the analysis for retinol, tocopherol, and β-carotene in three lyophilized sera, as analyzed at our laboratory and at the National Bureau of Standards, Gaithersburg, MD. The results obtained compare well and, although we have no comparable data for the other three carotenoids, we used the same methods for them as were used for β-carotene.

We incorporate the anti-oxidant BHT into the heptane extraction solvent as a precaution against oxidation losses during extraction and evaporation. Although we have no direct evidence that the BHT is, in fact, doing this, we are able to measure α-carotene in almost all our samples, even though it is a very minor carotenoid component. Milne and Botnen (12) reported no advantage in the use of BHT, but the ratio of BHT to serum they used was less than 2.5% of that reported here, which may be important.

Accuracy is also influenced by the resolution of the components and the specificity of the detection wavelengths. Carotenes do absorb at 325 and 292 nm, but the concentration is too low to do more than contribute a small amount of baseline noise at these wavelengths. β-Cryptoxanthin elutes just before α-tocopherol, but mixtures of appropriate concentrations of the individual standards (e.g., 0.2 and 20 μmol/L, respectively) do not interfere at the respective wavelengths (292, 450 nm). However, in analysis for the carotenoids, closely eluting peaks may interfere with the analysis of β-cryptoxanthin (Figure 1B), lycopene, and β-carotene (Figure 1C). The peak preceding β-cryptoxanthin has been termed pre-cryptoxanthin, and that following β-carotene is called 15,15′-cis-β-carotene (18), but the others are unknown. These closely eluting peaks are separately integrated from the carotenoids of interest, but small day-to-day variations in resolution may have a small effect on accuracy.

Time of separation. In most extracts, the last main peak to be eluted is β-carotene (Figure 1B), which makes the method particularly useful for providing fast, clean separations in approximately 6.5 min. Routinely, one sample can be analyzed every 8 min. Occasionally, the procedure had to be slightly modified to account for an additional substance eluted between 9 and 11 min in a very few samples. This substance absorbed at 292 nm and interfered with the absorbance of tocopherol and tocopherol acetate in the following sample. It was first detected in extracts from blood of Nigerian children with sickle cell disease and has subsequently been noticed in blood from two normal Nigerian children and in a noncommercial, lyophilized quality-control material prepared from human blood. It has not, however, been detected in any survey or hospital sample from adults or children living in the U.K. It may reflect the different dietary habits of Nigerians, whose carotene profile

Fig. 1. (A) Serum extract (25 μL) from a healthy U.K. adult, monitored on channel 1 at 325 nm for retinol (0.93 min) and 292 nm for α-tocopherol (2.15 min) and tocopherol acetate (2.61 min); the arrow indicates the time of wavelength change. (B) The same serum extract monitored on channel 2 at 450 nm: retention times (minutes) are shown for the peaks that have been identified: lutein (0.92), β-cryptoxanthin (2.07), lycopene (3.51), α-carotene (5.20), and β-carotene (5.67). (C) Serum (10 ml) from a healthy Nigerian child extracted and monitored as in B, showing a far higher proportion of both α- and β-carotene in the extract and shorter retention times associated with higher ambient temperatures than in A and B.

Response set at 1.0 A (A) or 0.05 A full-scale (B and C).
(Figure 1C) is very different from that observed in English subjects. Recent examination of fresh blood from Nigerians with sickle-cell disease failed to reveal the substance, which we now believe is an artifact caused by age or deterioration of the samples.

D.I.T. is grateful for continued support from the Department of Health & Social Security, U.K., and for the assistance of Hoffmann-La Roche, Switzerland, who supplied us with β-cryptoxanthin. We also thank Dr. F. Khachik for the sample of \( \text{C}_{45}\beta \)-carotene and Dr. R. Schaffer, National Bureau of Standards, Gaithersburg, MD, for allowing us to take part in the Fat-Soluble Vitamins Quality-Control Scheme.

References


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