THE DETERMINATION OF VITAMIN A AND CAROTENE WITH THE PHOTOELECTRIC COLORIMETER*

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The Carr-Price reaction (1) has been employed for a considerable period of time in testing biological materials for vitamin A, and it is generally accepted that the amount of blue color produced by the reaction of vitamin A with antimony trichloride is proportional to the amount of vitamin A present. The instability of the resulting blue color, however, has definitely limited the application of the reaction for the quantitative determination of vitamin A. Recently Dann and Evelyn (2) have shown that this limitation can be overcome by the use of a direct reading photoelectric colorimeter (3).

On the basis of spectrophotometric and colorimetric readings on a series of oils and concentrates these investigators (2) obtained a factor of 0.41 ± 0.05 for converting $I_{1\text{cm}}^{1\%} (620 \text{ m}\mu)$ into $E_{1\text{cm}}^{1\%} (328 \text{ m}\mu)$. The question of the factor to be used in converting $E_{1\text{cm}}^{1\%} (328 \text{ m}\mu)$ into biological units of vitamin A, however, is at present unsettled. Although the factor 1600, recommended by the League of Nations Conference on Vitamin Standards (1934), has been widely used for converting $E_{1\text{cm}}^{1\%} (328 \text{ m}\mu)$ into biological units of vitamin A, Mead, Underhill, and Coward (4) using crystalline vitamin A esters have recently arrived at a factor of 2000.

Since the factor for converting $E_{1\text{cm}}^{1\%} (328 \text{ m}\mu)$ into biological units of vitamin A is unsettled, we have calculated the units of vitamin A per 100 g of oil or concentrate from the spectrophotometric and colorimetric readings by using the factor 1600 for $E_{1\text{cm}}^{1\%} (328 \text{ m}\mu)$. The results are shown in Table I.

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1 The term $L_{1\text{cm}}^{1\%} (620 \text{ m}\mu)$ proposed by Dann and Evelyn (2) for use in photoelectric colorimetry is analogous to $E_{1\text{cm}}^{1\%} (620 \text{ m}\mu)$ used in spectrophotometry.
units of vitamin A is subject to controversy, it was decided to determine directly the relationship between \( L_{1\%}^{1500} \) (620 m\( \mu \)) and biological activity rather than use the indirect spectrophotometric basis. For this purpose the \( L_{1\%}^{1500} \) (620 m\( \mu \)) of the U.S.P. reference cod liver oil was determined and the potency of the reference oil was checked by biological assay against \( \beta \)-carotene.

Since the photoelectric colorimeter also affords a rapid means of determining carotene, and it is often desirable to determine carotene and vitamin A in the same solution, the \( L_{1\%}^{1500} \) (440 m\( \mu \)) of \( \beta \)-carotene was determined in chloroform and Skellysolve solutions. A correction factor was also determined for the amount of light absorbed by the blue color produced by the reaction of carotene with antimony trichloride.

Preliminary vitamin A determinations made on halibut liver oil and on the unsaponifiable matter of cod liver oil indicated that \( 2 - \log G^2 \) was not a strictly linear function of the concentration of vitamin A. Similar results were obtained with \( \beta \)-carotene in Skellysolve and chloroform solutions. The values of \( 2 - \log G \) were therefore determined over a wide range of concentrations of these chromogens.

**EXPERIMENTAL**

**Methods**

*Preparation of Reagent*—For the preparation of the antimony trichloride reagent, chloroform (Merck, reagent) was thoroughly washed with distilled water, dried over anhydrous potassium carbonate, and distilled in an all-glass still under reduced pressure at 30-35°, the first 25 ml. of the distillate being discarded. Antimony trichloride (Merck, reagent) was quickly added from freshly opened bottles; solution was effected by shaking the mixture at room temperature. The above procedures were carried out in the dark as much as possible. The reagent was siphoned into the reservoir of an automatic pipette which was designed to deliver 9.0 ml. of reagent in less than 2 seconds. The concentration of the reagent used in these studies was approximately 225 gm. per liter.

*The density of the sample solutions is expressed as \( 2 - \log G \), where \( G \) is the corrected galvanometer reading. The terms \( 2 - \log G_{620} \) and \( 2 - \log G_{440} \) specifically designate the density of the sample solutions when Filters 620 and 440, respectively, are used.*
The chloroform used as a solvent for the samples was merely
dried over potassium carbonate and filtered through sintered
glass. It was protected from moisture and light at all times.
The chloroform used in making up the antimony trichloride
reagent was found to be unsuitable as a solvent for the vitamin A
concentrates because of the rapid destruction of vitamin A by
the phosgene which was invariably formed in the alcohol-free
chloroform. Phosgene formation did not take place in the reagent,
because the antimony trichloride exerted a stabilizing influence on
the chloroform.

Peroxide-free ether was prepared by treating commercial ether
with sodium bisulfite as described by Smith (5). Aldehyde-free
alcohol was prepared by reduction with zinc and sodium hydroxide
as described by Dubovitz (6).

Method for Determination of Vitamin A—A 0.3020 gm. sample
of U.S.P. reference cod liver oil was weighed into a 50 ml. Erlen-
meyer flask to which were added 10 ml. of aldehyde-free alcohol
and 2.0 ml. of 60 per cent aqueous KOH; the mixture was placed
on a water bath and boiled for 2 minutes. The saponified solution
was cooled and transferred to a separatory funnel with 40 ml. of
distilled water and 50 ml. of peroxide-free ether. The contents
were thoroughly shaken and allowed to separate. The aqueous
layer was drawn off into another separatory funnel and again
extracted with 25 ml. of ether. The combined ether extracts were
washed once with 15 ml. and twice with 35 ml. of water. The
ether layer was dried over anhydrous sodium sulfate and
decanted; the sodium sulfate was washed three times with 10 ml.
of ether. The washings were combined with the ether extract in
a special evaporating flask which could be simultaneously con-
ected to a vacuum pump and a nitrogen cylinder. This was
placed on a water bath at 40° and the ether was removed under
reduced pressure in the presence of a gentle stream of nitrogen.
The last traces of moisture were driven off by dipping the flask for
several seconds in a 70° water bath. When the residue was
thoroughly dry, the stop-cock leading to the vacuum pump was
closed and the nitrogen stream continued until a slight positive
pressure of nitrogen was obtained in the flask. The nitrogen line
was then disconnected from the evaporator and chloroform was
introduced. The unsaponifiable residue was diluted to a 25 ml.
volume with chloroform. Eleven aliquots in duplicate of this
Vitamin A and Carotene solution ranging from 0.15 to 1.0 ml. were transferred to absorption test-tubes by means of a micropipette, diluted to 1.0 ml. each with chloroform, and the tubes tightly stoppered. The light intensity in the colorimeter was adjusted to give a galvanometer reading of 100 when a blank containing 1.0 ml. of chloroform and 9.0 ml. of antimony trichloride reagent was used. Each tube was then placed in the colorimeter and exactly 9.0 ml. of antimony trichloride reagent were added from the automatic pipette. The galvanometer was read at the point of temporary stability, Filter 620 being used. The galvanometer readings were corrected and the corresponding values of \(2 - \log G\) were recorded.

Method for Determination of \(\beta\)-Carotene—A Skellysolve solution (Skellysolve B, redistilled, b. p. 68-70°) of S. M. A. \(\beta\)-carotene containing 13.00 micrograms of \(\beta\)-carotene per ml. was used as a stock solution in the calibration of the photoelectric colorimeter for the determination of \(\beta\)-carotene. Twelve aliquots in duplicate of this solution were diluted to 10 ml. with Skellysolve in volumetric flasks. The exact concentration of these solutions was determined with a universal spectrophotometer, the value 2290 being used for the extinction coefficient, \(E_{1\%}^{1\text{cm.}} (475\text{ m\mu})\), of a Skellysolve solution of \(\beta\)-carotene. This same series of solutions was then read on the photoelectric colorimeter against a blank of pure Skellysolve, Filter 440 being used. The galvanometer readings were recorded and the values of \(2 - \log G\) corresponding to the corrected galvanometer readings were obtained. In order to determine carotene in chloroform solution a measured amount of the stock solution of \(\beta\)-carotene in Skellysolve was evaporated to dryness in an evaporator by the method described above. The carotene was then dissolved in chloroform and diluted to the same volume. Twelve aliquots in duplicate ranging from 0.10 to 2.00 ml. were measured out and diluted to 10 ml. as before. The solutions were then read against a blank of pure chloroform with Filter 440 and the values of \(2 - \log G\) were determined.

Correction for Presence of \(\beta\)-Carotene in Vitamin A Determina-

\(^2\) The photoelectric colorimeter used was of the Evelyn type, manufactured by the Rubicon Company of Philadelphia. Filters 620 and 440 used in the determination of vitamin A and carotene, respectively, were the standard filters supplied with the instrument and recommended by Dann and Evelyn (2) for these determinations.
tion—A stock solution of β-carotene dissolved in chloroform containing 14.1 micrograms of carotene per ml. was used in the determination. Aliquots ranging from 0.1 to 0.7 ml. were measured out with a micropipette and diluted to 1 ml. in the absorption test-tubes. Each tube was placed in the colorimeter and 9.0 ml. of antimony trichloride reagent were added from the automatic pipette. The values of $2 - \log G$ were determined, Filter 620 being used. A tube containing 1.0 ml. of chloroform and 9.0 ml. of reagent was used for a blank.

Method of Biological Assay—The method of biological assay described by Sherman (8) was used for determining the vitamin A potency of the U.S.P. reference cod liver oil.

S. M. A. β-carotene was used for comparison in the assay of the U.S.P. reference cod liver oil. The crystals were dissolved in Skellysolve and diluted to suitable volume so that the selected daily dose of 2 micrograms of carotene, as determined spectrophotometrically, was contained in 0.2 ml. The carotene solutions were stored in the dark at 3°.

Fresh samples of U.S.P. reference cod liver oil, with an assigned vitamin A potency of 3000 U.S.P. XI units per gm., were weighed and diluted to volume with Skellysolve. The solutions used in feeding contained 5.555 gm. of the reference cod liver oil per liter of solution and were likewise stored in the refrigerator. The U.S.P. reference cod liver oil was fed in Skellysolve solution at two levels, Level A to supply 3.33 units of vitamin A based on the assigned value of 3000 units per gm. (equivalent to 2 micrograms of β-carotene), and Level B to supply 3.33 I.U. based on the apparent potency calculated from the $L_{10}^{1\%}$ (620 mµ) determined with the photoelectric colorimeter. 4

4 Numerous vitamin A determinations were made with the photoelectric colorimeter on the unsaponifiable matter from two samples of U.S.P. reference cod liver oil at various concentrations. The average $L_{10}^{1\%}$ (620 mµ) of the oil was found to be 3.45. If this value is multiplied by 0.41, the factor obtained by Dann and Evelyn for converting $L_{10}^{1\%}$ (620 mµ) into extinction coefficients, the value of $E_{10}^{1\%}$ (328 mµ) = 1.414 is obtained. If this figure is multiplied by 1600, the factor recommended by the League of Nations Conference on Vitamin Standards (1934) for converting $E_{10}^{1\%}$ (328 mµ) into I.U. of vitamin A, the value of 2263 I.U. per gm. is obtained for the U.S.P. reference cod liver oil. In Level B the U.S.P. reference cod liver oil was fed to supply 3.33 I.U. calculated on this basis.
The carotene content of the solutions used in feeding was determined spectrophotometrically at biweekly intervals. The $L_{1\%}^{1\text{cm}}$ (620 mp) of the cod liver oil solutions was determined on the photoelectric colorimeter at biweekly intervals. For this purpose the solvent was removed under reduced pressure from a measured amount of the solution, and the determination was made on the unsaponifiable matter. It was found that the destruction of $\beta$-carotene at this concentration in Skellysolve solution stored at 3° in the absence of light was only about 0.5 per cent in 30 days. The vitamin A content of the cod liver oil stored in the same manner in Skellysolve solution decreased 2.3 per cent in 30 days.

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gains of Vitamin A-Deficient Rats Receiving $\beta$-Carotene and U.S.P. Reference Cod Liver Oil</strong></td>
</tr>
<tr>
<td>Supplement fed daily</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>$\beta$-Carotene (2 γ)</td>
</tr>
<tr>
<td>Cod liver oil, Level A*</td>
</tr>
<tr>
<td>&quot; &quot; &quot; &quot; B†</td>
</tr>
</tbody>
</table>

* 3.33 U.S.P. units of vitamin A based on the assigned value of 3000 U.S.P. units per gm. for the U.S.P. reference cod liver oil.  
† 3.33 I.U. of vitamin A based on the determined $L_{1\%}^{1\text{cm}}$ (620 mp) of 3.45 and the factors 0.41 and 1600 for converting $L_{1\%}^{1\text{cm}}$ (620 mp) into I.U. of vitamin A. See foot-note 4 to the text.

The aliquots used for feeding were corrected for any slight deterioration that was observed.

Results

*Potency of U.S.P. Reference Cod Liver Oil As Determined by Biological Assay*—The results of the biological assay of the U.S.P. cod liver oil are given in Table I. It is apparent that the average gain obtained when the reference oil was fed at Level A, a more concentrated solution of several samples of S. M. A. crystalline carotene and S. M. A. $\beta$-carotene in Skellysolve (400 to 500 micrograms per ml.) showed from 11 to 40 per cent destruction in 30 days storage at 3° in the dark.
to supply 3.33 U.S.P. units of vitamin A based on the assigned value of 3000 U.S.P. units per gm., is in agreement with that obtained from 2 micrograms of β-carotene (equivalent to 3.33 units of vitamin A). The average gain of the rats receiving the U.S.P. reference cod liver oil at Level B is appreciably greater than that obtained with 2 micrograms of β-carotene or with the reference cod liver oil at Level A. The assigned value of 3000 U.S.P. units of vitamin A per gm. for the U.S.P. reference cod liver oil was therefore accepted for the calibration of the photoelectric colorimeter.

**Table II**

Variation of 2 - Log G with Concentration of Vitamin A

\[ K_1 = 45.60 \]

<table>
<thead>
<tr>
<th>Galvanometer reading (corrected)</th>
<th>2 - log G</th>
<th>2 - log G per I.U. vitamin A</th>
<th>Vitamin A in test solution</th>
<th>Per cent error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>international</td>
<td>international</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>units</td>
<td>units</td>
<td></td>
</tr>
<tr>
<td>91°</td>
<td>0.0410</td>
<td>0.0226</td>
<td>1.81</td>
<td>+3.32</td>
</tr>
<tr>
<td>83°</td>
<td>0.0815</td>
<td>0.0225</td>
<td>3.62</td>
<td>+2.76</td>
</tr>
<tr>
<td>68°</td>
<td>0.1619</td>
<td>0.0224</td>
<td>7.25</td>
<td>+1.89</td>
</tr>
<tr>
<td>57°</td>
<td>0.2422</td>
<td>0.0223</td>
<td>10.87</td>
<td>+1.47</td>
</tr>
<tr>
<td>47°</td>
<td>0.321</td>
<td>0.0222</td>
<td>14.49</td>
<td>+0.97</td>
</tr>
<tr>
<td>40°</td>
<td>0.398</td>
<td>0.0220</td>
<td>18.12</td>
<td>+0.11</td>
</tr>
<tr>
<td>34°</td>
<td>0.471</td>
<td>0.0217</td>
<td>21.75</td>
<td>-1.24</td>
</tr>
<tr>
<td>28°</td>
<td>0.543</td>
<td>0.0214</td>
<td>25.39</td>
<td>-2.48</td>
</tr>
<tr>
<td>24°</td>
<td>0.620</td>
<td>0.0214</td>
<td>28.99</td>
<td>-2.48</td>
</tr>
<tr>
<td>21°</td>
<td>0.683</td>
<td>0.0210</td>
<td>32.61</td>
<td>-4.39</td>
</tr>
<tr>
<td>17°</td>
<td>0.760</td>
<td>0.0209</td>
<td>36.24</td>
<td></td>
</tr>
</tbody>
</table>

**Relation between 2 - Log G and Concentration of Vitamin A and Carotene**—The results given in Table II show that 2 - log G is not strictly a linear function of the concentration of vitamin A, but that a distinct decrease in 2 - log G per I.U. of vitamin A occurred as the concentration of vitamin A was increased in the test solution. Similar results were obtained with β-carotene in chloroform and Skellysolve solutions as shown in Table III. It is evident that constants for converting 2 - log G into units of vitamin A and micrograms of β-carotene are applicable only over a limited range of concentrations. Such a constant, \[ K_1 = 45.60 \], was derived by
averaging the values obtained by dividing the concentration of vitamin A, expressed as I. U. present in the test solution, by the corresponding $2 - \log G$ of these solutions over the galvanometer range from 30 to 70. Similar constants, $K_2 = 3.19$ and $K_3 = 2.65$, were derived for converting $2 - \log G$ into micrograms of $\beta$-carotene per ml. in chloroform and Skellysolve solutions respectively. When these constants were applied, the $L_{\text{1cm}}^{\text{1%}}$ (440 m\(\mu\)) of $\beta$-carotene was found to be 1645 and 1980 in chloroform and Skellysolve solutions respectively.

### Table III

**Variation of $2 - \log G$ with Concentration of $\beta$-Carotene in Chloroform and Skellysolve**

<table>
<thead>
<tr>
<th>$2 - \log G$</th>
<th>$\beta$-Carotene</th>
<th>Per cent error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chloroform</td>
<td>Skellysolve</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Skellysolve</td>
<td>$\gamma$ per ml.</td>
</tr>
<tr>
<td>0.0434</td>
<td>0.0506</td>
<td>0.130</td>
</tr>
<tr>
<td>0.0848</td>
<td>0.0996</td>
<td>0.260</td>
</tr>
<tr>
<td>0.1264</td>
<td>0.1487</td>
<td>0.390</td>
</tr>
<tr>
<td>0.1659</td>
<td>0.1973</td>
<td>0.520</td>
</tr>
<tr>
<td>0.2460</td>
<td>0.2760</td>
<td>0.780</td>
</tr>
<tr>
<td>0.3300</td>
<td>0.3870</td>
<td>1.04</td>
</tr>
<tr>
<td>0.4010</td>
<td>0.478</td>
<td>1.30</td>
</tr>
<tr>
<td>0.478</td>
<td>0.565</td>
<td>1.56</td>
</tr>
<tr>
<td>0.542</td>
<td>0.636</td>
<td>1.82</td>
</tr>
<tr>
<td>0.602</td>
<td>0.710</td>
<td>2.08</td>
</tr>
<tr>
<td>0.663</td>
<td>0.770</td>
<td>2.34</td>
</tr>
<tr>
<td>0.710</td>
<td>0.834</td>
<td>2.60</td>
</tr>
</tbody>
</table>

The galvanometer range of 30 to 70 was selected because the percentage variation of $2 - \log G$ with the galvanometer readings is less over this range than at either end of the scale.

If the concentration of chromogen in the test solution is adjusted so that a galvanometer reading between 30 and 70 is obtained, an error of less than 2 per cent is incurred by the use of constant $K_1$ and less than 3 per cent by the use of constants $K_2$ and $K_3$ as shown in Tables II and III. Beyond this range the error is greater, especially at the higher concentrations. If calibration curves, made by plotting concentration of vitamin A and carotene against
the corresponding values of $2 - \log G$ are used, these errors are eliminated.

The variation of $2 - \log G$ with the amount of blue color produced by the reaction of antimony trichloride with various concentrations of $\beta$-carotene is shown in Table IV. The series of dilutions used covers the range of carotene usually encountered in the determination of vitamin A in biological materials. Since the range of galvanometer readings was necessarily small, the constant $K_4 = 156.3$ was calculated for the entire range from 87 to 98 and is the average of the values obtained by dividing the number of

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\beta-carotene in test solution & Galvanometer reading (corrected) & $2 - \log G_{20}$ & $2 - \log G_{20}$ & Per cent error \\
\hline
$\gamma$ & $\gamma$ per ml. & & & & \\
\hline
1.05 & 98 & 0.0084 & 0.0088 & 0.0067 & -23.82 \\
1.41 & 97 & 0.0078 & 0.0110 & 0.0090 & -18.18 \\
2.82 & 95 & 0.0071 & 0.0200 & 0.0180 & -10.00 \\
4.23 & 93 & 0.0069 & 0.0292 & 0.0271 & -7.23 \\
5.64 & 91 & 0.0066 & 0.0374 & 0.0361 & -3.48 \\
7.05 & 90 & 0.0063 & 0.0446 & 0.0451 & +1.12 \\
8.46 & 88 & 0.0061 & 0.0518 & 0.0541 & +4.44 \\
9.87 & 87 & 0.0057 & 0.0568 & 0.0631 & +11.09 \\
\hline
\end{tabular}
\caption{Application of Correction Factor $K_4 = 156.3$ for Amount of Blue Color Produced by Reaction between $\beta$-Carotene and Antimony Trichloride}
\end{table}

$* C =$ micrograms of $\beta$-carotene in the test solution.

micrograms of carotene in the test solution by the corresponding $2 - \log G$. The error involved by the use of this constant is given in Table IV.

\textit{Calculation of Results}—The following calculations are involved if the constants given in this paper are to be used to convert the values of $2 - \log G$ into micrograms of $\beta$-carotene or I.U. of vitamin A.

$(2 - \log G_{20})K_1 = \text{I.U. of vitamin A in the 10 ml. test solution (uncorrected for the presence of carotene)}$ where $K_1 = 45.60$.

$(2 - \log G_{45})K_2 = \text{micrograms of } \beta \text{-carotene per ml. in the 10 ml. test solution (chloroform)}$ where $K_2 = 3.19$. 

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\[(2 - \log G_{440})K_3\] micrograms of \(\beta\)-carotene per ml. in the 10 ml. test solution (Skellysolve) where \(K_3 = 2.65\).

\[(2 - \log G_{440})K_3/K_4 = \text{correction factor to be subtracted from } 2 - \log G_{620} \text{ for the amount of the blue color produced by the reaction of antimony trichloride with the } \beta\text{-carotene in the } 10 \text{ ml. test solution where } K_4 = 156.3. \] When \(K_3\) and \(K_4\) are combined the following equation is obtained.

\[(2 - \log G_{440})K_3/ \text{correction factor for } \beta\text{-carotene where } K_6 = 0.0204.\]

\[[(2 - \log G_{620}) - (2 - \log G_{440})K_6]K_1 = \text{I.U. of vitamin A in the } 10 \text{ ml. test solution (corrected for the presence of carotene).}\]

DISCUSSION

Although the photoelectric colorimeter affords a rapid and reliable means of determining carotene and vitamin A, there are certain inherent limitations in its use. It was found that \(2 - \log G\) for vitamin A and \(\beta\)-carotene is not a strictly linear function of the concentration of the chromogens. It is therefore apparent that constants for converting \(2 - \log G\) into units of vitamin A and micrograms of carotene are not absolutely accurate. To obtain a high degree of accuracy with the instrument, a calibration curve should be used in which the values of \(2 - \log G\) are plotted against the concentration over a series of dilutions of the material to be determined. By employing such a calibration curve the errors resulting from the non-linear relationship between the values of \(2 - \log G\) and the concentration may be completely avoided.

When such a high degree of precision is not essential, however, conversion factors may be used without introducing serious errors provided the concentration of chromogen in the test solution is such as to give a galvanometer reading over a limited range. The constants for vitamin A and carotene given above are for solutions of such concentration as to give galvanometer readings between 30 and 70; their use results in an error of less than 3 per cent. Constants to cover a still narrower range of the galvanometer scale would produce appreciably less error.

The observation that a straight line relationship does not exist between \(2 - \log G\) and concentration of vitamin A and \(\beta\)-carotene does not confirm the work of Dann and Evelyn (2). Shrewsbury,
Kraybill, and Withrow (9), however, using a photoelectric photometer of their own design (10), observed that density was not a linear function of the concentration of \( \beta \)-carotene. These workers concluded that the non-linearity was due to the relatively wide spectral band which the filters they used transmitted. Filter 440 used in the work reported in the present paper had a transmission band of from 410 to 475 m\( \mu \) and Filter 620 had a transmission band of from 505 to 660 m\( \mu \). It is apparent that an instrument employing a filter with a transmission band of 65 m\( \mu \) will not give the degree of linearity which can be obtained with a spectrophotometer in which a spectral band of only a few m\( \mu \) can be selected to correspond exactly to an absorption maximum of the compound.

On the basis of the results of the biological assays and the chemical determinations of vitamin A made on the U.S.P. reference cod liver oil and the factor 0.41 of Dann and Evelyn (2), a factor of 2120 was derived for the conversion of \( E_{1\text{cm}, 328 \text{ m\( \mu \)}} \text{ of } \beta \text{-carotene} \) into I.U. of vitamin A. This is in close agreement with the value of 2150 given by Mead, Underhill, and Coward (4) as a result of their work with vitamin A-2-naphthoate. The figure 1440 given by Dann and Evelyn (2) for the \( L_{1\text{cm}, 440 \text{ m\( \mu \)}} \text{ of } \beta \text{-carotene} \) is somewhat lower than the value given in this paper. They state, however, that their value was undoubtedly too low because of the impurity of the \( \beta \)-carotene used. Although the S. M. A. \( \beta \)-carotene used in the work reported here was not absolutely pure, the exact concentration of \( \beta \)-carotene in the solutions was determined spectrophotometrically. It is believed, therefore, that \( L_{1\text{cm}, 440 \text{ m\( \mu \)}} = 1645 \) is near the correct value for \( \beta \)-carotene in chloroform solution.

**SUMMARY**

1. The U.S.P. reference cod liver oil when assayed biologically in comparison with \( \beta \)-carotene was found to have the assigned potency of 3000 I.U. of vitamin A per gm. This same oil was found to have an average \( L_{1\text{cm}, 620 \text{ m\( \mu \)}} \text{ of } 3.45 \) as determined with the Evelyn photoelectric colorimeter.

2. Calculations are presented for converting colorimeter readings into I.U. of vitamin A and micrograms of carotene. Constant \( K_1 = 45.60 \) was derived for converting \( 2 - \log G_{620} \) into I.U. of vitamin A, and constants \( K_2 = 3.19 \) and \( K_3 = 2.65 \) were derived
for converting $2 - \log G_{440}$ into micrograms of $\beta$-carotene in chloroform and Skellysolve solutions respectively. A correction factor was also derived for the light absorbed by the blue color produced in the reaction between $\beta$-carotene and antimony trichloride.

3. It was found that $2 - \log G$ was not a strictly linear function of concentration of vitamin A or carotene. The constants given were calculated for concentrations giving galvanometer readings from 30 to 70. If these constants are used within this range, an error of less than 3 per cent is incurred. For more accurate work it is recommended that a calibration curve be used in which $2 - \log G$ is plotted against concentration of the chromogen.

4. $\beta$-Carotene was found to have an average $L_{1\%}^{1\%}$ (440 m$\mu$) of 1645 and 1980 in chloroform and Skellysolve solutions respectively.

5. On the basis of the biological assays, the determined $L_{1\%}^{1\%}$ (620 m$\mu$) of 3.45 for the u.s.p. reference cod liver oil, and factor 0.41 for converting $L_{1\%}^{1\%}$ (620 m$\mu$) into $E_{1\%}^{1\%}$ (328 m$\mu$), a factor of 2120 was obtained for converting $E_{1\%}^{1\%}$ (328 m$\mu$) into I.U. of vitamin A per gm.

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