The Uptake of C\textsuperscript{14}-\(\beta\)-Carotene and Its Conversion to Retinol Ester (Vitamin A Ester) by the Isolated Perfused Rat Liver* 

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The small intestine is probably the major site for the conversion of \(\beta\)-carotene into retinol ester (vitamin A ester\textsuperscript{1}) in the mammal (2-5), but other tissues are capable of catalyzing this cleavage reaction, e.g., the conversion takes place \textit{in vivo} after removal of the intestine (6) and of various other organs (6-9). Direct evidence for the participation of other tissues, however, is lacking. Although the concentrations of \(\beta\)-carotene and retinol in the lung increase \textit{in vivo} after the injection of \(\beta\)-carotene (10), practically no \(\beta\)-carotene was converted into retinol ester in the dog heart-lung preparation (11).

As early as 1931, Olcott and McCann (12) claimed that ground liver tissues contained the enzyme "carotenase," which converted carotene into retinol. However, Woolf and Moore (13), with the use of more careful analytical methods, were unable to repeat these experiments. With a variety of techniques, others (14-19) supported the view that carotene could be converted into retinol in liver tissues. On the other hand, conversion did not occur in rat liver perfused with carotene suspended in gum-NaCl (20), and other experiments with liver tissue (21-24) failed to demonstrate the conversion. Moore (25) has critically reviewed these studies.

Since C\textsuperscript{14}-\(\beta\)-carotene has been used to demonstrate retinol ester formation in the rat intestine both \textit{in vivo} (5) and \textit{in vitro} (26), labeled \(\beta\)-carotene was employed in investigating the role of the liver in \(\beta\)-carotene metabolism. In the present communication, the conversion of \(\beta\)-carotene into retinol ester by an isolated perfused rat liver is clearly demonstrated.

**Experimental Procedure**

The C\textsuperscript{14}-\(\beta\)-carotene was isolated from \textit{Phycomyces blakesleeanus} grown on C\textsuperscript{14}-acetate (27), was crystallized three times to constant specific activity, and was chromatographed on deactivated alumina just before use. Two preparations had specific activities of 2850 and 4000 c.p.m. per \(\mu\)g. Given amounts, 10 to 25 \(\mu\)g, of C\textsuperscript{14}-\(\beta\)-carotene were solubilized in 0.05 ml of Tween 80 and Krebs-Ringer bicarbonate buffer, pH 7.4.

Male rats (Rolismeyer Farm, Madison, Wisconsin) which weighed 200 to 300 g were used. The perfusion apparatus was like that designed by Green and Miller (28). A variation of the procedure of Brauer, Pessotti, and Pizzolato (29) for removal of the liver and its perfusion was employed. With the liver still in situ, the vena cava was cut and the portal vein was ligated some 5 to 4 cm from its entrance into the liver. Immediately thereafter 8 to 10 ml of flushing solution (oxygenated Krebs-Ringer bicarbonate buffer, pH 7.4, which contained 1% citrate and 0.5% glucose) at 37° were injected into the portal vein. After cannulation of the portal vein, 75 to 100 ml of flushing solution were immediately passed through the liver, thereby removing nearly all traces of blood. With this procedure, the liver was left without oxygen and fluid flow for only two short periods of about 2 minutes each. The liver was then removed and placed in the perfusion apparatus. The perfusion fluid was 98 ml of 0.5% glucose in Krebs-Ringer bicarbonate buffer, pH 7.4, which had been equilibrated at 38° with 95% O\textsubscript{2}-5% CO\textsubscript{2}. The pressure head on the liver was maintained at 18 to 23 cm of water, which gave a flow rate of 70 to 90 ml per minute. This flow rate was established within 3 to 7 minutes, and no period of pronounced vasoconstriction was noted. The flow of 95% O\textsubscript{2}-5% CO\textsubscript{2} through the glass lung was 1500 ml per minute. After 8 to 10 minutes of perfusion, 2.0 ml of the \(\beta\)-carotene solution were added to the perfusate reservoir, 98 ml.

In order to measure \(\beta\)-carotene uptake, samples of 2.0 ml were taken from the perfusate at appropriate times. Duplicate aliquots were plated, dried, and counted for a total of 5,000 or 10,000 counts in a windowless gas flow Geiger-Müller counter. The observed counts per minute were corrected for self-absorption.

At the conclusion of the experiment, the liver was removed from the perfusion apparatus, flushed with 50 to 75 ml of 0.9% NaCl, washed, blotted dry, weighed, and homogenized for 15 minutes in 100 ml of hexane-100% ethanol (3:1) containing 100 \(\mu\)g of unlabeled carotene and 300 \(\mu\)g of unlabeled retinol acetate as carriers. After filtration, washing with 20 ml of 2% CaCl\textsubscript{2} solution, and evaporation, the lipid extract in hexane was chromatographed on deactivated alumina. The fractions obtained with a given eluent, in the order of their elution, are as follows: \(\beta\)-carotene (hexane), retinol ester (additional hexane), retinal (2% acetone in hexane), retinol (5% acetone in hexane), and terminal polar fractions (100% acetone and glacial acetic acid).
Conversion of \( \beta \)-carotene into retinol ester by isolated perfused rat liver

Perfusion with \( ^{14} \mathrm{C} \)-\( \beta \)-carotene in the amounts indicated was carried out for 2 hours. The liver lipids were then extracted, chromatographed, and assayed as described in the text. The 100% acetone fraction (polar fraction) includes all substances with similar or greater polarity than retinal and retinol, but does not include the glacial acetic acid fraction.

Table I

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>( ^{14} \mathrm{C} )-( \beta )-carotene added to perfusate</th>
<th>Disappearance rate constant</th>
<th>Liver weight</th>
<th>Liver ( ^{14} \mathrm{C} ) components</th>
<th>100% Acetone fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>c.p.m.</td>
<td>( \mu )l</td>
<td>( \text{min}^{-1} )</td>
<td>g</td>
<td>c.p.m.</td>
</tr>
<tr>
<td>3-4</td>
<td>32,000</td>
<td>11.2</td>
<td>5.6 ( \times ) 10(^{-3} )</td>
<td>10.2</td>
<td>4000</td>
</tr>
<tr>
<td>3-7</td>
<td>65,000</td>
<td>22.7</td>
<td>5.6 ( \times ) 10(^{-1} )</td>
<td>10.2</td>
<td>6600</td>
</tr>
<tr>
<td>3-9</td>
<td>60,000</td>
<td>21.0</td>
<td>4.0 ( \times ) 10(^{-2} )</td>
<td>9.5</td>
<td>7220</td>
</tr>
</tbody>
</table>

The final perfusate was diluted with an equal volume of 95% ethanol, 2 volumes of hexane containing carrier \( \beta \)-carotene and retinol acetate were added, and the mixture was shaken for 6 to 8 minutes. The hexane phase was dried with Na\( \text{SO}_4 \), concentrated, and chromatographed on alumina.

In several cases, rigorous characterization of the liver \( ^{14} \text{C} \)-retinol ester was carried out. Pooled ester fractions from the alumina column were concentrated under vacuum, saponified under \( \text{N}_2 \) at 60° for 1 hour with 15% KOH in 95% methanol, extracted with hexane, and the retinol was isolated by chromatography on alumina. This retinol was then converted to the semicarbazone. Details of the extraction, chromatography, and characterization procedures have been published (5).

The average first order rate constant for 10 experiments was \( 5.6 \times 10^{-3} \pm 0.51 \times 10^{-3} \text{ min}^{-1} \), with values ranging from \( 4.6 \times 10^{-3} \text{ min}^{-1} \) to \( 6.8 \times 10^{-3} \text{ min}^{-1} \). The recovery of radioactivity after 2 hours of perfusion was 70 to 90%; 22 to 30% of the total radioactivity was found in the liver and 50 to 60% remained in the perfusate.

Results

Extent of \( \beta \)-Carotene Uptake—The uptake of \( \beta \)-carotene by the liver is first order, at all levels of \( \beta \)-carotene used (10 to 70 \( \mu \)g) and throughout all time periods employed (30 to 120 minutes). The average first order rate constant for 10 experiments was \( 5.6 \times 10^{-3} \pm 0.51 \times 10^{-3} \text{ min}^{-1} \), with values ranging from \( 4.6 \times 10^{-3} \text{ min}^{-1} \) to \( 6.8 \times 10^{-3} \text{ min}^{-1} \). The recovery of radioactivity after 2 hours of perfusion was 70 to 90%; 22 to 30% of the total radioactivity was found in the liver and 50 to 60% remained in the perfusate.

Metabolic Products of \( \beta \)-Carotene in Liver—Of the 18 to 24% of the total radioactivity found in the hexane phase of liver extracts after 2 hours of perfusion, the \( \beta \)-carotene fraction contained 10 to 13% of the original radioactivity, retinol ester had 6 to 9%, and more polar fractions, such as retinal, retinol, etc., contained approximately 1.5 to 2.5% (Table I). In addition, the aqueous \( \text{CaCl}_2 \) wash of the hexane phase of liver homogenates had 4 to 6%. After 2 hours of perfusion with 20 \( \mu \)g of \( \beta \)-carotene, the amount of \( \beta \)-carotene in the liver was approximately 1.5 times greater than the amount of retinol ester. The formation of retinol ester by the liver was linear with perfusion time from 30 to 120 minutes (Fig. 1). When the initial concentration of \( \beta \)-carotene in the perfusate was 20 to 22 \( \mu \)g per 100 ml and the liver wet weight was 8 to 10 g, 0.6 to 0.8 \( \mu \)g of retinol ester was formed per hour. The rate of retinol ester formation was also linearly dependent on the initial concentration of \( \beta \)-carotene in the perfusate up to 30 to 40 \( \mu \)g per 100 ml, but plateaued when higher \( \beta \)-carotene concentrations were used (Fig. 2). The maximal observed rate of retinol ester formation was 1.3 \( \mu \)g per hour.

On several occasions, the retinol ester fraction was hydroyzed and a series of derivatives was made. Typical results are shown in Table II. The specific activities of retinol ester and subsequent derivatives remained fairly constant. The final recovery of radioactivity in retinal semicarbazone was usually 20 to 30% of that originally present in the retinol ester fraction. Owing to the removal of aliquots, however, the counts per minute given in Table II are less than those actually recovered.

After elution of \( \beta \)-carotene and retinol ester from the alumina...
columns, 100% acetone was generally used to elute more polar products from the column. When a more complete chromatographic separation was carried out, the retinal, retinol, and polar fractions each contained 0.5 to 1.0% of the original perfusate radioactivity; a glacial acetic acid fraction usually contained 0.2 to 0.5%. The major portion of these more polar fractions probably contains oxidized derivatives of \( \beta \)-carotene rather than retinol, retinal, or retinoic acid. The radioactivity in these fractions was not isolated and characterized.

**Distribution of Radioactivity in Perfusate**—The distribution of radioactivity in the hexane extract of the perfusate after 2 hours of perfusion was: \( \beta \)-carotene, 33 to 90%; ester fraction, <1%; retinal fraction, 3 to 5%; retinol fraction, 3 to 5%; acetone-glacial acetic acid fraction, 5 to 8%. The ester fraction contained too little radioactivity for further characterization. The retinal fraction did not seem to contain retinal itself. Radioactivity in the retinal fraction could be eluted from deactivated alumina with 1% acetone, whereas retinal requires 2% acetone. Similarly the retinol fraction contained little retinol, if any. After oxidation of the retinol fraction of the perfusate with \( \text{MnO}_2 \), the specific activity of the resultant retinal decreased to 20 to 25% of that of the retinol fraction. In other experiments, the retinal and retinol fractions were contaminated in a similar way by autoxidation products of \( \beta \)-carotene when \( \beta \)-carotene was incubated under oxygen with inactivated intestinal segments. Hence, very little retinal and retinol are present in the final perfusate.

**Effect of Sodium Glycocholate**—Since sodium glycocholate at 0.2 to 0.4% maximally stimulates the absorption and cleavage of \( \beta \)-carotene into retinol ester in the rat intestine (5, 35), its effect on the liver conversion system was examined. At bile salt concentrations of 0.0002 to 0.002%, similar to those found in rat portal blood (36), no change was observed in the rate of \( \beta \)-carotene uptake, in the amount of retinol ester formed, or in the distribution of components in the liver (Table III). At higher glycocholate concentrations (0.1 to 0.4%) the amount of \( \beta \)-carotene present in the liver extracts increased markedly but retinol ester formation decreased to 30 to 40% of normal. The rate constant for \( \beta \)-carotene disappearance at 0.4% glycocholate was 16 to 18 \( \times 10^{-3} \) min\(^{-1}\), 3 to 4 times greater than normal. In these cases, the liver became whitish and edematous after a few minutes of perfusion and was far from normal in appearance.

**Effect of Serum and Bovine Serum Albumin**—The addition to the perfusate of rat serum, obtained by cardiac puncture of rats deprived of food for 12 to 15 hours, had a definite effect on \( \beta \)-carotene uptake and cleavage (Fig. 3). At lower concentrations (<7%) the total uptake of \( \beta \)-carotene (total radioactivity) was unaffected but the formation of retinol ester was lowered. As the concentration of serum in the perfusate was increased from 7 to 30%, the total uptake of \( \beta \)-carotene and its amount in liver decreased, whereas the formation of retinol ester increased slightly and then decreased. Dialysis of serum before use did not alter the effect at the 2% level.

Rovine serum albumin at concentrations of 0.15%, 1%, and 2.4% decreased the amounts of \( \beta \)-carotene and retinol ester in the liver (Fig. 4). The rate constants for \( \beta \)-carotene uptake were 4.0 \( \times 10^{-3} \) min\(^{-1}\) at 0.15% bovine serum albumin, 3.3 \( \times 10^{-3} \) min\(^{-1}\) at 1%, and 3.0 \( \times 10^{-3} \) min\(^{-1}\) at 2.4%.

**TABLE II**

**Characterization of retinol ester**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Compound</th>
<th>Counts per minute</th>
<th>Mg</th>
<th>Specific activity (counts per minute per mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-9</td>
<td>Retinol ester</td>
<td>5300</td>
<td>1.56</td>
<td>3400</td>
</tr>
<tr>
<td></td>
<td>Retinol</td>
<td>2600</td>
<td>0.81</td>
<td>3280</td>
</tr>
<tr>
<td></td>
<td>Retinal</td>
<td>1680</td>
<td>0.51</td>
<td>3300</td>
</tr>
<tr>
<td></td>
<td>Retinal semi-</td>
<td>1150</td>
<td>0.45</td>
<td>2550</td>
</tr>
<tr>
<td></td>
<td>carboxyzone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-21</td>
<td>Retinol ester</td>
<td>4250</td>
<td>1.18</td>
<td>3600</td>
</tr>
<tr>
<td></td>
<td>Retinol</td>
<td>2000</td>
<td>0.67</td>
<td>3000</td>
</tr>
<tr>
<td></td>
<td>Retinal</td>
<td>1800</td>
<td>0.47</td>
<td>3800</td>
</tr>
<tr>
<td></td>
<td>Retinal semi-</td>
<td>1120</td>
<td>0.32</td>
<td>3500</td>
</tr>
<tr>
<td></td>
<td>carboxyzone</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

Rat liver can convert \( \beta \)-carotene into retinol ester. The clear demonstration of this capability has depended largely on the methods employed. With the exception of early reports (12, 15-17), attempts to demonstrate the conversion of \( \beta \)-carotene into retinol by slices or homogenates of liver have not been successful (13, 21-23, footnote 2). Hence, most past studies (6-8, 18-19) on retinol formation in liver have not been successful because of the difficulty in defining the specific role of a given organ in vivo. The use of an isolated perfused liver in our studies has allowed more precise definition of its metabolic activities under conditions approaching those in vivo.

The liver in our perfusion system functions well. During its surgical isolation and transfer to the apparatus, the liver is subjected to only two short periods of possible anoxia. Flow rates of the oxygenated perfusate are high from the initiation of the experiment, and the color, general macroscopic appearance, and...
Effect of sodium glycocholate on \( \beta \)-carotene uptake and conversion by isolated perfused rat liver

\( \text{C}^{14} \beta \)-carotene and sodium glycocholate were present in the perfusate in the amounts indicated. The experimental period was 1 hour. Livers were then extracted and the hexane extract treated as described in the text.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Glycocholate</th>
<th>( \text{C}^{14} )-( \beta )-carotene added to perfusate</th>
<th>Disappearance rate constant</th>
<th>Liver weight</th>
<th>Liver ( \text{C}^{14} ) components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>c.p.m.</td>
<td>( \mu g )</td>
<td>\text{min}^{-1}</td>
<td>( e )</td>
</tr>
<tr>
<td>3-17</td>
<td>0</td>
<td>60,000</td>
<td>21.0</td>
<td>( 6.6 \times 10^{-3} )</td>
<td>9.7</td>
</tr>
<tr>
<td>6-16</td>
<td>0</td>
<td>46,000</td>
<td>10.0</td>
<td>( 6.2 \times 10^{-3} )</td>
<td>11.0</td>
</tr>
<tr>
<td>3-15</td>
<td>0.0002</td>
<td>69,000</td>
<td>24.2</td>
<td>( 6.8 \times 10^{-3} )</td>
<td>11.3</td>
</tr>
<tr>
<td>3-14</td>
<td>0.0004</td>
<td>53,000</td>
<td>18.0</td>
<td>( 6.2 \times 10^{-3} )</td>
<td>8.2</td>
</tr>
<tr>
<td>3-18</td>
<td>0.0006</td>
<td>64,000</td>
<td>22.5</td>
<td>( 5.8 \times 10^{-3} )</td>
<td>8.7</td>
</tr>
<tr>
<td>3-19</td>
<td>0.0020</td>
<td>57,000</td>
<td>20.0</td>
<td>( 5.3 \times 10^{-3} )</td>
<td>10.4</td>
</tr>
<tr>
<td>6-19</td>
<td>0.02</td>
<td>46,000</td>
<td>10.0</td>
<td>( 4.4 \times 10^{-3} )</td>
<td>9.0</td>
</tr>
<tr>
<td>6-18</td>
<td>0.1</td>
<td>46,000</td>
<td>10.0</td>
<td>( 4.9 \times 10^{-3} )</td>
<td>10.0</td>
</tr>
<tr>
<td>6-18</td>
<td>0.4</td>
<td>46,000</td>
<td>10.0</td>
<td>( 15.6 \times 10^{-2} )</td>
<td>10.5</td>
</tr>
<tr>
<td>6-16</td>
<td>0.4</td>
<td>46,000</td>
<td>10.0</td>
<td>( 18.2 \times 10^{-2} )</td>
<td>9.0</td>
</tr>
</tbody>
</table>

The characterization of retinol derivatives in the presence of \( \beta \)-carotene and its autoxidation products requires care, as several investigators have stressed (11, 21, 25). In the present case, special attention was paid to this problem. Fortunately, the retinol ester fraction, unlike more polar fractions, is initially contaminated to a slight degree only, and the radioactivity present in this fraction from liver was 10 to 20 times the expected amount of contaminant. Furthermore, the specific activities of a series of retinol derivatives remained relatively constant under conditions in which autoxidation products of \( \beta \)-carotene were rapidly eliminated. The 800-fold difference between the specific

![Figure 3](http://www.jbc.org/)

**Fig. 3.** Effect of serum on \( \beta \)-carotene metabolism in the perfused liver. \( \beta \)-Carotene (60,000 c.p.m. in 21 \( \mu g \)) was suspended in 0.05 ml of Tween 80 and added to the perfusate which contained the indicated amounts of serum. The total perfusate volume was 100 ml, and the perfusion period was 2 hours. \(-\)-, retinol ester; \( \Delta \)-\( \Delta \), \( \beta \)-carotene; \( \bullet \)-\( \bullet \), 100% acetone fraction; \( \circ \)-\( \circ \), total radioactivity.

The microscopic structure of the liver are normal during the perfusion period. Bile flow and the clearance of Bromsulfalein and carbon particles by these livers are satisfactory. In addition, for a large group of perfused livers, the first order rate constant for the disappearance of \( \beta \)-carotene from the perfusate is reproducible and the rate of retinol ester formation is linear. The use of \( \text{C}^{14} \)-\( \beta \)-carotene of high specific activity has also been advantageous in several respects: the formation of small amounts of retinol ester is readily detected and measured, the retinol ester formed can be identified and characterized with some rigor, and use of small amounts (10 to 25 \( \mu g \)) of \( \beta \)-carotene, amounts that are easily solubilized in detergents and that might appear in plasma under physiological conditions, avoids the complications that arise when high concentrations of \( \beta \)-carotene are employed. In other studies (12, 15, 17, 20, 23, 24) 30 to 1000 times as much \( \beta \)-carotene has been used.

**Fig. 4.** Effect of bovine serum albumin on \( \beta \)-carotene uptake and metabolism by perfused rat liver. \( \beta \)-Carotene (42,000 to 46,000 c.p.m.) in 0.05 ml of Tween 80 was added to the perfusate which contained various amounts of bovine serum albumin. The perfusion period was 90 minutes. \(-\)-\( \circ \), retinol ester; \( \Delta \)-\( \Delta \), \( \beta \)-carotene; \( \square \)-\( \square \), 100% acetone fraction; \( \bullet \)-\( \bullet \), total radioactivity.

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\* R. D. Zachman, unpublished observations.
activities of the \( \beta \) carotene in the perfusate, 2500 c.p.m. per \( \mu \)g, and of the retinol derivatives, 3.5 c.p.m. per \( \mu \)g, given in Table II can be readily explained. The 1.6 to 1.8 \( \mu \)g of \( C^{14} \)-retinol ester formed from \( C^{14} \)-\( \beta \) carotene in these experiments is diluted by the 300 \( \mu \)g of carrier retinol acetate added during the extraction procedure and by approximately 750 \( \mu \)g of endogenous retinol in the 10 g of perfused liver (32). Hence, the total calculated dilution of the radioactive retinol is 600- to 700-fold, which is in reasonable agreement with the observed value.

Thus, at least two organs of the rat, the liver and the intestine (2-5, 37), are capable of converting \( \beta \)-carotene into retinol. Of the two, the intestine is the more active; it is approximately twice as active per whole organ and approximately 4 to 7 times as active per g of tissue, wet weight. Nevertheless, the liver of a 200-g rat formed 0.6 to 0.8 \( \mu \)g of retinol ester per hour under our perfusion conditions, approximately 3 times the amount required for full longevity in the rat (5, 38). Thus, in animals like the rat, in which \( \beta \)-carotene does not readily pass the intestinal mucosa into the plasma, the liver probably plays a minor physiological role in the formation of retinol. In other species, such as man and cow, in which plasma carotenoid levels are high, both the liver and intestine are probably involved in \( \beta \)-carotene cleavage.

Conjugated bile salts are required for the uptake of \( \beta \)-carotene and its cleavage into retinol ester in rat intestine (5, 38). Although taurocholic acid is the most common bile salt in rats (39), sodium glycocholate and several other conjugated bile salts are equally effective. Neither at the concentrations of bile salt found in portal blood (0.0002 to 0.002\%\( \)) nor at concentrations found to stimulate intestinal uptake and cleavage (0.1 to 0.4\%), however, was glycocholate required for the conversion of \( \beta \)-carotene into retinol ester in the perfused liver, nor did glycocholate enhance retinol ester formation. Neither was the uptake of \( \beta \)-carotene affected by low concentrations of bile salts. Thus, the action of bile salts in enhancing the cleavage of \( \beta \)-carotene into retinol ester is seemingly limited to the intestine. At 0.4\% sodium glycocholate, the uptake of \( \beta \)-carotene increased markedly, although retinol ester formation was depressed. In view of the apparent liver damage that occurred in these experiments, it is difficult to ascertain whether the increased uptake of \( \beta \)-carotene is directly due to the glycocholate present, or is indirectly caused by a nonspecific adsorption of the micellar particles on degenerating tissue elements. With intestinal tissue, the amount of \( \beta \)-carotene adsorbed is inversely related to the integrity and metabolic activity of the tissue.2

Protein enhances the uptake and release of fatty acids by tissues (40, 41), the induction of pinocytosis and phagocytosis (42, 43), and perhaps the release of triglycerides from tissues (41). On the other hand, protein depresses the removal of colloidal chromium phosphate particles from blood (44) and is not necessary for triglyceride uptake in perfused liver (45). In plasma, carotene is bound in lipoprotein complexes (46, 47) and proteins have been postulated to be necessary carriers of carotenoids and retinol in plasma (48). In our studies, however, no requirement for protein in the uptake of carotene and its conversion into retinol ester could be shown. Maximal rates of uptake and conversion occurred when \( \beta \)-carotene was suspended only in a nonionic surface active agent, Tween 80. Since the livers were well cleared of blood before perfusion, the protein concentration of the perfusate was very low. Furthermore, the addition of rat serum or bovine serum albumin to the perfusate did not stimulate \( \beta \)-carotene uptake and retinol ester formation, but rather inhibited them. Whether proteins stabilize plasma carotene by forming specific complexes or inhibit the mechanism of uptake has not been defined. Protein might also be important for the release of retinol from liver, since retinol is associated with protein in plasma (49). However, the release of retinol derivatives from the liver during perfusion could not be demonstrated, even in the presence of serum. Clearly, factors other than serum protein must be involved in the release mechanism. These may be of a hormonal nature (50).

In view of the fact that \( \alpha \)-tocopherol inhibits the conversion into retinol ester of \( \beta \)-carotene and of a series of \( \beta \)-apo-carotenals injected intravenously into intact rats as aqueous dispersions in Tween 40, McGillivray (51) has suggested that the cleavage of \( \beta \)-carotene into \( \beta \)-apo-carotenals occurs in the blood, and perhaps is catalyzed nonspecifically by hemoglobin or hemat in. No intermediates could be isolated from blood, however, and the formation of retinol ester from \( \beta \)-carotene was greater than from the \( \beta \)-apo-carotenals. In our study, in which the rate of formation of retinol ester is similar to that reported by McGillivray, blood was absent or was present only in traces. Therefore, blood is certainly not required for \( \beta \)-carotene cleavage to retinol ester, and its involvement in \( \beta \)-carotene cleavage in the intact animal seems unlikely.

Serum, unlike bovine serum albumin at similar protein concentrations, had an anomalous effect on the \( \beta \)-carotene and retinol ester concentrations in liver. At serum concentrations to 5\%, liver \( \beta \)-carotene increased and retinol ester fell markedly, which suggests that serum contains a factor that inhibits \( \beta \)-carotene cleavage to retinol ester in liver. However, at 10 to 20\% serum, \( \beta \)-carotene decreased greatly whereas more \( C^{14} \)-retinol ester was formed than at 2 to 5\% serum. Since serum contains no \( \beta \)-carotene but does have appreciable amounts of retinol, the radioactivity in the retinol ester fraction of liver may decrease as a result of dilution of the retinol pool. Furthermore, retinol at high concentrations inhibits \( \beta \)-carotene cleavage in the intestine (5). The higher conversion of carotene into retinol ester at 10\% serum than at 5\% serum, however, rules out any simple explanation of these effects. Whether serum affects the micelle containing \( \beta \)-carotene in a critical way or contains specific inhibitors and activators remains to be established.

**SUMMARY**

1. \( C^{14} \)-\( \beta \)-carotene is converted into retinol ester by the isolated perfused rat liver. The amount of retinol ester formed was directly proportional to the time of perfusion, 0.6 to 0.8 \( \mu \)g per hour at initial \( \beta \)-carotene concentrations of 20 to 22 \( \mu \)g per 100 ml of perfusate, and was dependent on the initial concentration of \( \beta \)-carotene in the perfusate

2. The kinetics of the uptake of \( \beta \)-carotene was first order for experimental periods up to 2 hours \(( k \sim 5.6 \times 0.811 \times 10^{-4} \text{ min}^{-1})\), with no evidence of a saturating effect at the levels of \( \beta \)-carotene used (10 to 70 \( \mu \)g).

3. In several cases the retinol ester fractions were characterized by hydrolysis to the alcohol, oxidation of the alcohol to the aldehyde, and formation of the semicarbazone of the aldehyde. The specific activities of these derivatives remained relatively constant.

4. Sodium glycocholate had no effect on the uptake or conversion of \( \beta \)-carotene in this perfused liver system at concentrations to 0.1\%, but at a concentration of 0.4\%, \( \beta \)-carotene uptake...
inhibited. Liver damage was evident at 0.4% glycocholate.

5. Bovine serum albumin and serum from rats deprived of food inhibited the uptake of β-carotene and its conversion into retinol ester.

REFERENCES

The Uptake of C$^{14}$-β-Carotene and Its Conversion to Retinol Ester (Vitamin A Ester) by the Isolated Perfused Rat Liver

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