CAROTENASE. THE TRANSFORMATION OF CAROTENE TO VITAMIN A IN VITRO*

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The work of Moore, Capper, and Drummond and their co-workers (3–5, 11) has definitely established the role of carotene as a precursor of vitamin A. When carotene, or substances containing it, were fed to vitamin A-free rats or fowls, the livers of such animals were found to contain vitamin A. Moore (12) showed that the liver contains more vitamin A than other tissues, and concluded that the conversion takes place in that organ. If an enzyme, carotenase, is responsible for this transformation, it should be possible to prepare vitamin A from carotene in vitro by incubation with whole liver or liver extracts. It is the purpose of this paper to outline a series of experiments which demonstrate that such a reaction does take place.

EXPERIMENTAL

In the present study the presence of vitamin A was detected by ultra-violet absorption spectra methods, since vitamin A is characterized by a broad absorption band with a maximum near 328μ (2, 6, 8, 13, 14, 18). A Hilger E-2 quartz spectrograph in conjunction with a sector photometer and a tungsten-steel spark was used for the photographs.

The absorption curves of carotene, achoorcarotene (16), and carotene decolorized by oxidation are presented in Fig. 1. These curves agree with those of Duliere, Morton, and Drummond (7), Capper (2), and McNicholas (10) for carotene and oxidized carotene. The absence of a band at 328μ indicates that decoloriza-

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tion of carotene by heat or oxidation does not yield vitamin A, an observation which has been confirmed by biological assays. It should be noted that carotene has bands at $280\mu m$ and $345\mu m$, and no band at $328\mu m$, and that its absorption is greater than either of its derivatives as indicated by the concentration used in the cell.

![Absorption spectrum of carotene](image)

**FIG. 1.**—absorption spectrum of carotene in chloroform, 1:75,000; achroocarotene in chloroform, 1:50,000; oxidized carotene in chloroform, 1:10,000.

Fresh whole liver from vitamin A-free rats was used in the first attempts to produce vitamin A *in vitro*. The rats had been fed on a diet consisting of sucrose (46 per cent), lard (24 per cent), extracted casein (18 per cent), dried yeast¹ (8 per cent), salts² (4 per cent)

¹ Courtesy of the Northwestern Yeast Company.
per cent), and viosterol\(^8\) (10 drops per kilo of diet). When a rat became deficient in vitamin A, as denoted by a rapid loss of weight, it was killed by a blow on the head, and its liver extirpated.

The liver from Rat 347 (Fig. 2) weighed 5 gm. It was thoroughly ground with sand, 15 cc. of a phosphate buffer solution at pH 7.45, and approximately 2 cc. of ethyl laurate in which had been dissolved 2 mg. of carotene (from lettuce (15)). The mixture was allowed to stand 24 hours at 38\(^\circ\), 10 cc. of 10 per cent potassium hydroxide were added, and the incubation continued for another 24 hours. The saponified material was then thoroughly extracted with ether, the ether was washed, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was dissolved in approximately 300 times its weight of chloroform, and the absorption spectrum photographed. A

\[\text{Fig. 2. Growth curves of rats on vitamin A-free diet. Rat 333 was fed 0.010 mg. of carotene per day for 8 days before it was killed.}\]
distinct band at 328\(\mu\) (Fig. 3) indicated that vitamin A was present. Although the ether extract had been slightly yellow, there was not sufficient carotene remaining to give the typical carotene bands. The same procedure was followed in two more experiments with similar results.

Fig. 3. ——— absorption spectrum of unsaponifiable liver lipids of Rat 347, carotene added, in chloroform, 1:300; ———— same of Rat 331, control, in chloroform, 1:300; ——— same of Rat 333, previously fed carotene, in chloroform, 1:500.
The liver from Rat 331 (Fig. 2) was treated in the same way except that the ethyl laurate added contained no carotene. The absorption spectrum (Fig. 3) showed no indication of a band at 328\(\mu\), nor did the extracts from two more livers treated similarly absorb in this region. Capper (3) was likewise unable to distinguish a band at 328\(\mu\) in the absorption spectra of liver oils from vitamin A-depleted rats.

Two rats were allowed to become depleted of vitamin A stores; they were then fed carotene dissolved in ethyl laurate, and when growth had become rapid, they were killed and their livers examined for vitamin A. The spectra of both oils were similar; to save space, as in the previous cases, the protocol of only one is included. The liver of Rat 333 (Fig. 2) was cut into small pieces and dissolved in 10 per cent potassium hydroxide by warming for 24 hours. The saponified mixture was extracted as in the previous cases, and the absorption of the extracted material determined. The band at 328\(\mu\) (Fig. 3) was again evident, confirming the observations of Moore, Capper, and others (3, 5, 11) that carotene was changed in the body to vitamin A, and from the similarity of the absorption curves, justifying the assumption that the band found after the \textit{in vitro} incubation of carotene with liver tissue was due to vitamin A.

An active extract of carotenase was prepared in the following manner: The livers from Rats 293 and 320 (Fig. 2) were combined, thoroughly ground with sand and 50 cc. of toluene-water, and the mixture allowed to incubate for 24 hours at 37\(^\circ\). The digest was then filtered through cheese-cloth and coarse filter paper, and the filtrate reserved as a solution of the enzyme. 2 cc. of the liver extract were thoroughly extracted with ether. The ether was evaporated to dryness, the residue dissolved in a small amount of chloroform, and its absorption spectrum determined as before (Fig. 4). The absence of a band at 328\(\mu\) indicated that the extract contained no appreciable amounts of vitamin A.

A colloidal solution of carotene in water was prepared by the method suggested by Fodor and Schoenfeld (9). A stable colloid, exhibiting a marked Tyndall effect, was obtained which contained approximately 0.02 mg. of carotene per cc. 6 cc. of this solution were mixed with 2 cc. of the liver extract, and allowed to incubate for 36 hours. The ether extract was colorless, indicating that the
Carotenase

carotene had been changed during the incubation. An absorption spectrum of the residue in chloroform showed a band at 328\( m\mu \) (Fig. 4).

2 cc. of the liver extract were heated to boiling, cooled, the solution of carotene added, and the incubation allowed to proceed exactly as before. The ether extract after incubation was yellow, but the color faded during the separate manipulations, and the concentration of the remaining carotene was not sufficient to give the typical carotene spectrum. The absence of the band at 328\( m\mu \) (Fig. 4) demonstrated the thermolability of the agent responsible for the reaction.

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**FIG. 4.** Absorption spectrum of ether-soluble constituents of liver extract, in chloroform; --- ether-soluble constituents of liver extract after incubation with carotene in chloroform; --- ether-soluble constituents of heated liver extract after incubation with carotene, in chloroform.
DISCUSSION

Von Euler and his coworkers (8) have recently shown that dihydrocarotene retains some of the growth-promoting property of carotene; further, that a mixture of the more fully hydrogenated products has three absorption bands in the ultra-violet region, one of which has a maximum at 328 m\(\mu\). Their results suggest that vitamin A may be related to reduced carotene, from which it follows that the function of carotenase might be that of catalyzing a particular type of reduction. Bruins, Overhoff, and Wolff (1) determined the molecular weight of vitamin A by a comparison of the diffusion constants of carotene and the vitamin. From their results, they deduce a molecular weight of 330 (carotene, mol. wt., 536) and state that "the value obtained causes the assumption of a simple chemical relation between vitamin A and carotene to appear improbable." It is possible that vitamin A is a reduced fragment of the carotene molecule, and that reduction of carotene itself creates that part of the molecule responsible for the absorption at 328 m\(\mu\).

Previous workers have noted a band at 280 m\(\mu\) in the absorption spectra of cod liver oils (13, 17), and Capper (3) called attention to inflections in that region in the absorption spectra of rat liver oils. The bands were in no case as pronounced as those observed in the present study. Both carotene (Fig. 1) and its isomer, lycopin (unpublished observations), have a band at 280 m\(\mu\), although lycopin differs from carotene in lacking the band at 345 m\(\mu\). One of the three important bands of ergosterol is at 280 m\(\mu\). Although it may be a chance occurrence, it is possible that some molecular configuration, common to carotene, lycopin, ergosterol, and the unknown substances in fish and animal oils, is responsible for the absorption at 280 m\(\mu\). The character of the compounds present in liver which contain the configuration absorbing at 280 m\(\mu\) remains to be determined. Whatever compound is responsible, it seems to be present in equal amounts in the separate rat livers (Fig. 3).

The concentration of the solutions used to determine the absorption curves presented in Fig. 4 was not known since the amount of material used was too small to be weighed; consequently the heights of the bands are not proportional to the total amount of absorbing substance (a condition which would be true if the
same concentration of the separate oils had been used). A more exact comparison of the vitamin A content can be obtained by superimposing the peaks of the 280μ bands on one another, that is, by assuming that each 2 cc. of liver extract contained an equal amount of the unknown substance absorbing at 280μ. If such a comparison is made with the curves in Fig. 3, it will be seen that the liver treated with carotene in vitro contained considerably less vitamin than that of the rat previously fed carotene.

Further study of the absorption curves in Figs. 3 and 4 reveals that, by using a liver extract, it has been possible to reduce the relative amount of the unknown substance. The control curve of the liver extract falls off less sharply than the control curve of the whole liver, and the vitamin A content is proportionately several times greater in the experiments with liver extract.

**SUMMARY**

Carotene can be changed to vitamin A by incubation with fresh liver tissue or with an aqueous extract of liver. The agent responsible for the transformation appears to be an enzyme, provisionally called carotenase, since it is destroyed by heat. The conversion of carotene to vitamin A in vivo and the usefulness of ultra-violet absorption spectrum methods for the detection of vitamin A are confirmed.

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**BIBLIOGRAPHY**
