Preparation of β-carotene-^{14}C

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In response to a need for relative large amounts of highly labeled ^{14}C β carotene, a search was made for a biological system which would supply an adequate amount of carotene with sufficient label. Methods previously described did not appear to be adequate [1,2]. Mated cultures of *Blakeslea trispora* [3] appear to devote a greater part of their metabolism to the synthesis of β carotene than do other systems. The results of growing mated cultures of *Blakeslea trispora* in the presence of $^{14}CO_2$, U^{14}C glucose (Nuclear Chicago) * and 1-2^{14}C acetic acid (Nuclear Research Chemicals) are reported.

Blakeslea trispora 2895 and *2896*, obtained from Northern Utilization Research Development Division, ARS, U. S. Department of Agriculture, were carried on potato dextrose agar slants. Five to 10 day old cultures were planted into 150 ml sterile 5% pharmamedia * and 0.5% yeast extract in 500 ml erlenmyer flask stoppered with cotton. The flasks were incubated on a rotary shaker at 200 strokes per minute, 28-32° C for 48 hours. At that time 5 ml of each culture were transferred to 100 ml of sterile media in 500 ml flasks. The medium consisted of 7% Pharmamedia, 5% deodorized kerosene, 0.5% yeast extract, and 5% lard. These flasks were incubated under the same conditions as the seed cultures. At the end of 72 hours 0.5% of sterile β ionone and the radioactive compound were added after sterilization by membrane filtration.

All of the labeled compounds used gave metabolic $^{14}CO_2$. To trap the $^{14}CO_2$, a special plug was made for the flasks. A double layer of cheesecloth 6 × 6 inches was placed over the mouth of an empty flask and pushed into the neck about 1 1/2 inches. A 1/4 inch layer of cotton was pushed to the bottom of the pocket and covered with another layer of cheesecloth. The plug was sterilized in the empty flask by autoclaving. At the time radioactive compounds were put in the incubation flasks, the plug was transferred to the incubation flask. About 5 g of Ascarite were placed in the cavity of the plug and covered with cotton to hold the Ascarite in place during shaking. The Ascarite was changed after 48 hours of subsequent incubation.

* Use of trade names of specific material does not constitute a recommendation by the U. S. Department of Agriculture to the exclusion of others which may also be available.

Seventy-two hours after adding the ionone and labeled compound, the flasks were opened under a hood, and 150 ml methanol were stirred into the medium. The carotenes were extracted and chromatographed as previously described [3]. The β carotene was crystallized once from hot methanol and twice from hot hexane. Specific radioactivity was determined by measuring the concentration of β carotene in hexane spectrophotometrically at 450 mμ and counting aliquots in a scintillation spectrometer [4]. Specific activity did not change in the last two crystallizations. The yields and specific radioactivities of β carotene-^{14}C obtained from the various substrates are given in Table I.

TABLE I. Incorporation of ^{14}C from Various Compounds into the β Carotene of *Blakeslea trispora*.

Source of label	Added radioactivity mC/flask	Yield of β carotene mg/flask	Radioactivity of β carotene mC/mM
$^{14}CO_2$	.5 mC	3.25	.020
Glucose-U-^{14}C	.25 mC	13.8	.024
Sodium Acetate-1,2-^{14}C	.5 mC	9.8	.846

Attempts were made to grow the mated cultures by stirring them with a magnetic stirrer under flowing atmospheres. This system was desired since it would provide positive trapping of metabolic $^{14}CO_2$. Good growth of the mold was obtained, but the yield of carotene was very low, 1-2 mg/100 ml media.

Substrates which offered the possibility of yielding β carotene labeled in all positions were chosen. Presumably dark fixed CO_2 would be so randomized in a growing culture that all carbons of the carotene would become labeled. Although this assumption has not been tested, there is little incentive for further study since sufficiently high specific radioactivity was not regularly achieved with $^{14}CO_2$. The same statements also apply to the use of glucose-U-^{14}C.

Sufficient levels of specific radioactivities were obtained from sodium acetate-1,2-^{14}C. It is reasonable to assume that all carbons of β carotene would be labeled under these conditions [2, 5, 6, 7].

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