INCORPORATION OF 2-C^{14}-MEVALONIC ACID INTO TOMATO CAROTENES*

David G. Anderson, David W. Norgard and John W. Porter

The Radioisotope Unit, Veterans Administration Hospital,
and the Department of Physiological Chemistry
University of Wisconsin, Madison, Wisconsin

Ripening tomatoes rapidly incorporate injected 2-C^{14}-mevalonic acid into non-saponifiable lipids. Chromatography on MgO - Super Cel columns of a petroleum ether extract of these compounds shows an association of carbon-14 with phytoene, phytofluene, β-carotene, ζ-carotene, γ-carotene, neurosporene and lycopene. However, in the case of each of these compounds at least 90% of the accompanying radioactivity is not present in the carotenoid. Separation of the contaminating radioactivity may be achieved by rechromatography on a different adsorbent or by crystallization. Thus β-carotene and lycopene are freed of radioactive contaminants through crystallization to constant specific radioactivity. Phytoene, phytofluene and ζ-carotene are separated from radioactive impurities through chromatography on alumina. Ethyl ether-petroleum ether mixtures are used in developing these columns. Neurosporene, γ-carotene and lycopene are freed of high counting contaminants by rechromatography on calcium hydroxide. The spectral purity of the eluted or crystallized carotenes and colorless polyenes was demonstrated with a Process and Instruments recording spectrophotometer. In the case of the eluted carotenoids, specific radioactivity measurements were made upon the series of eluates collected for each compound. Those fractions of similar or identical specific radioactivities were combined and further proof of the presence of C^{14} in each

* Presented in part at the Boston Meeting, American Chemical Society, April 1959. This investigation was supported in part by a research grant, A-1383, from the National Institute of Arthritic and Metabolic Diseases of the National Institutes of Health, United States Public Health Service.

of these compounds was obtained through catalytic hydrogenation of each. Crystalline, non-radioactive lycopene was added to each carotene or colorless polyene, i.e. phytoene, phytofluene, ζ-carotene and neurosporene, and the compound was catalytically hydrogenated to the derivative perhydrolycopene. The hydrogenated compound was then chromatographed on alumina. In each instance complete coincidence was obtained between weight and radioactivity. Coincidence was also obtained between radioactivity of reduced γ-carotene and weight of perhydrolycopene. The presence of C^{14} in these carotenoids is further evidenced by the formation of the thiourea adduct of perhydrolycopene (Rabourn and Quackenbush, 1956). All of the radioactivity appeared in the adduct. In all cases at least 90% or more of the carrier lycopene and 60% or more of the radioactivity of the purified carotenoid appeared in perhydrolycopene. The radioactivity present in the hydrogenated product was taken as the measure of radioactivity present in the original carotenoid. These values were used in calculating the specific radioactivities given in Table I.

The values clearly indicate that 2-C^{14}-mevalonic acid is incorporated into each of the carotenoid compounds listed, but much less effectively than into other non-saponifiable compounds synthesized by the tomato. The data of Table I are at variance, where comparisons can be made, with those of Purcell, Thompson and Bonner (1959) who did not present convincing evidence of a high degree of purity of most of the carotenoids which they separated.

Table I

SPECIFIC RADIOACTIVITIES OF TOMATO CAROTENOIDS

Compound	Total radioactivity[a] First chromatogram	Second chromatogram	After reduction	Specific radioactivity
	c/min.	c/min.	c/min.	c/m/mg.
Phytoene	2,060,000[b]	1295	1238	2870±200
Phytofluene	459,000[b]	1995[c]	1142	3080±200
ζ-carotene	24,300[b]	440[b]	310	750±100
Neurosporene	20,500[b]	—	45	780±150
γ-carotene	37,000[b]	284[b]	224	3450±250
	Before crystallization	After crystallization		
Lycopene	162,000[b]	18,400[b]	—	3110± 50[b]
β-carotene	242,000[b]	7,850[b]	—	6400±100[b]

[a] Radioactivity was measured with a packard Tri-Carb liquid scintillation spectrometer.

[b] Radioactivity was measured with a thin end window gas-flow Geiger-Muller tube, and values were corrected for comparison with those obtained with the liquid scintillation spectrometer.

[c] Radioactivity values were corrected for quenching.

REFERENCES

Purcell, A. E., Thompson, G. A., Jr., and Bonner, J., J. Biol. Chem., 234, 1081 (1959).

Rabourn, W. S., and Quackenbush, F. W., Arch. Biochem. Biophys., 61, 111, (1956).

Received August 6, 1959