Metabolism and Biological Potency of 5,6-Monoepoxy- β -Carotene and 5,6:5',6'-Diepoxy- β -Carotene

By C. SUBBARAYAN, M. R. LAKSHMANAN AND H. R. CAMA

Department of Biochemistry, Indian Institute of Science, Bangalore 12, India

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1. 5,6-Monoepoxy- β -carotene and 5,6:5',6'-diepoxy- β -carotene were partially converted into the furanoid forms during passage through the rat stomach. 2. The monoepoxide was converted into vitamin A in the small intestine and showed a biological potency 21% of that of β -carotene. Neither β -carotene nor 5,6-monoepoxyvitamin A was formed. 3. Intraperitoneal administration of the monoepoxide led to the accumulation of the unchanged compound in the liver and other tissues. 4. The diepoxy gave no β -carotene or vitamin A or 5,6-monoepoxyvitamin A when given orally and showed no biological potency. 5. The significance of these results with special reference to the mechanism of formation of vitamin A from β -carotene is discussed.

Previous studies in this Laboratory on the metabolism of 5,6-monoepoxyretinal in rat demonstrated that this compound is metabolized without giving rise to vitamin A anywhere in the body (Lakshmanan, Jungalwala & Cama, 1965); 5,6-monoepoxyretinal was also shown to be biologically as potent as all-*trans*-retinyl acetate. As these oxidized derivatives of vitamin A substituted in the β -ionone ring can not only replace the classical vitamin A in its systemic functions but also in vision (Lakshmanan & Cama, 1966), interest was aroused about the origin of these compounds *in vivo*. Since β -carotene serves as the natural precursor of vitamin A, the corresponding 5,6-epoxides of β -carotene may very well be the precursors of 5,6-monoepoxyvitamin A compounds. The chemical synthesis of 5,6-monoepoxy- β -carotene and 5,6:5',6'-diepoxy- β -carotene as well as the biological potency of the latter have been reported (Karrer & Jucker, 1945; Karrer & Euler, 1950; Tsukida & Zechmeister, 1958); however, the metabolism of these compounds has not been hitherto investigated and the mode of action and their biological activity is little understood.

MATERIALS AND METHODS

Materials. Synthetic all-*trans*- β -carotene and all-*trans*-retinyl acetate were a gift from Hoffmann-La Roche, Basle, Switzerland. 5,6-Monoepoxy- β -carotene and 5,6:5',6'-diepoxy- β -carotene were prepared according to the method of Tsukida & Zechmeister (1958) and the melting points and spectral characteristics were in good agreement with those reported by these authors.

Oral administration. The compounds were dissolved in refined deodorized groundnut oil containing 0.5% of α -tocopherol (unless otherwise stated) and were stored at

-20°. They were administered with the aid of an Agla micrometer syringe (Burroughs Wellcome). For bioassay experiments, 5,6-monoepoxy- β -carotene and β -carotene were freshly prepared (concentrations 2.5 and 5.0 μ g./0.05 ml. of oil) every week. For metabolic experiments, 5,6-monoepoxy- β -carotene and 5,6:5',6'-diepoxy- β -carotene were prepared just before use (1.0 mg./0.1–0.2 ml. of diluent oil containing 0.5 mg. of α -tocopherol).

Separation and identification of vitamin A and various epoxides of β -carotene. The compounds were separated on a 3%-(v/w)-water-deactivated alumina column. Various epoxides of β -carotene were separated and purified on a magnesium oxide-Celite (1:1, w/w) column (Tsukida & Zechmeister, 1958; Subbarayan, Jungalwala & Cama, 1965). Vitamin A was identified by its characteristic ultraviolet fluorescence, absorption maxima, SbCl₃ test and behaviour on thin-layer chromatography (John, Lakshmanan, Jungalwala & Cama, 1965). Various epoxides of β -carotene were identified as their specific coloured HgCl₂ complexes (Subbarayan *et al.* 1965) and by their reactions with ethanolic HCl (Goodwin, 1955).

Epoxides of β -carotene were estimated spectrophotometrically. Vitamin A was estimated by the SbCl₃ colour test by using the correction procedure of Cama, Collins & Morton (1951). Bioassay of 5,6-monoepoxy- β -carotene was carried out according to the more precise U.S.P. XIV method as described by Lakshmanan *et al.* (1965).

RESULTS

Oral administration of 5,6-monoepoxy- β -carotene. Two vitamin A-deficient rats were given 5,6-monoepoxy- β -carotene by mouth (1 mg./rat/day) for 7 days. The average weight gain of the two rats during this period was 10 g. They were killed 24 hr. after the last dose and the recoveries of the compounds from various tissues are recorded in Table 1. The lipid extracts from the contents of the intestinal

Table 1. Recovery of vitamin A, mutatochrome, luteochrome and aurochrome from various tissues of two vitamin A-deficient rats after oral administration of 5,6-monoepoxy- β -carotene and 5,6:5',6'-diepoxy- β -carotene for 7 days at a dose rate of 1.0mg./rat/day

Tissue	Recovery of 5,6-monoepoxy- β -carotene (%)		Recovery of 5,6:5',6'-diepoxy- β -carotene (%)		
	As vitamin A	As mutatochrome	As vitamin A	As luteochrome	As aurochrome
Control	—	—	—	—	—
Experimental					
Liver	3.03	0.39	—	—	2.0
Intestine	—	0.12	—	—	0.28
Intestinal washings	—	—	—	—	—
Stomach	—	—	—	0.59	—
Kidneys	—	—	—	—	—
Spleen	—	—	—	—	—
Lungs	—	—	—	—	—
Blood (serum)	—	—	—	—	—

washings, stomach, kidneys, spleen, lungs and serum failed to show any spectral evidence for the presence of 5,6-monoepoxy- β -carotene, 5,8-monoepoxy- β -carotene (mutatochrome) or vitamin A.

The extract from the small intestine gave the spectrum of 5,8-monoepoxy- β -carotene, which was further purified and identified. The extract from the liver gave the spectrum of vitamin A in the ultraviolet region and that of 5,8-monoepoxy- β -carotene in the visible region. The extract was chromatographed on a 3%-(v/w)-water-deactivated alumina column: the vitamin A fraction was eluted first, followed by the 5,8-monoepoxy- β -carotene fraction. No trace of either 5,6-monoepoxyvitamin A (Jungalwala & Cama, 1965) or β -carotene was detected.

To investigate the active absorption of 5,6-monoepoxy- β -carotene and its probable site of conversion into vitamin A, time-distribution studies were carried out with vitamin A-deficient rats. The results (Table 2) demonstrate that in the stomach during the first 3 hr. of absorption there is a rapid increase of 5,6-monoepoxy- β -carotene, which presumably is converted into its furanoid form during the next 9 hr. 5,8-Monoepoxy- β -carotene could also be detected in the intestine and liver between 6 and 12 hr. after administration. Vitamin A was detected in the intestine between 6 and 24 hr. after administration; it was gradually stored in the liver. However, the extent of conversion of 5,6-monoepoxy- β -carotene into vitamin A was only about 2.5%.

5,6:5',6'-Diepoxy- β -carotene. Two vitamin A-deficient rats were given 5,6:5',6'-diepoxy- β -carotene by mouth (1 mg./rat/day) for 7 days. These rats were not relieved of any vitamin A-deficiency symptoms and each rat showed a decline in weight of, on average, 3–4 g. during this period. As the

compound failed to support the growth of vitamin A-deficient rats, bioassay of this compound could not be carried out.

The rats were killed 24 hr. after the last dose and the various epoxides of β -carotene isolated from different tissues were identified and estimated (Table 1). The extracts from the contents of the stomach gave the characteristic absorption spectrum of luteochrome, which was purified by chromatography on an alumina column and identified by the ethanolic hydrochloric acid test as well as by the characteristic absorption maximum of the mercuric chloride complex.

The extracts from the intestine and liver gave the characteristic absorption spectrum of aurochrome, which was further purified and identified by its characteristic absorption maximum of the mercuric chloride complex. No trace of vitamin A, 5,6-monoepoxyvitamin A, β -carotene, 5,6-monoepoxy- β -carotene or 5,8-monoepoxy- β -carotene was detected in any of the tissues.

Intraintestinal administration. Rats were anaesthetized and, after an incision had been made along the abdomen, the middle one-third of the intestine was ligated at both ends. A 1.0 mg. sample of 5,6-monoepoxy- β -carotene was injected directly into the intestine just below the upper ligature, the abdominal incision was sutured and the animals were returned to their cages. They were killed after 4 hr. and the intestines between the two ligatures as well as the livers were analysed. Both the intestines and livers contained mostly 5,6-monoepoxy- β -carotene, but the total recovery of 5,6-monoepoxy- β -carotene was only 14.3%. Only traces of vitamin A could be detected in the intestine and liver. Intraintestinal administration of 5,6:5',6'-diepoxy- β -carotene resulted only in the destruction of the administered compound.

Table 2. Conversion of 5,6-monoepoxy- β -carotene into vitamin A in the intestine of the rat after oral administration of 1.0mg. of 5,6-monoepoxy- β -carotene

of 1.0 mg. of 5,6-monoepoxy- β -carotene												
Rat no.	Time between administration and death (hr.)	5,6-Monoepoxy- β -carotene recovered (μ g.)				Mutatochrome recovered (μ g.)				Vitamin A recovered (μ g.)		Total recovery of 5,6-monoepoxy- β -carotene + mutatochrome + vitamin A (% of dose)
		Stomach	Intestine	Intestinal washings	Liver	Stomach	Intestine	Intestinal washings	Liver	Intestine	Liver	
1 (control)	—	0	0	0	0	0	0	0	0	0	0	0
2 (control)	—	0	0	0	0	0	0	0	0	0	0	0
3	0.5	139.0	—	—	—	—	—	—	—	—	—	13.9
4	0.5	153.0	—	—	—	—	—	—	—	—	—	15.3
5	1	5.8	64.9	8.8	—	—	—	—	—	—	—	8.0
6	1	45.2	149.0	19.7	—	—	—	—	—	—	—	21.4
7	3	233.0	316.4	172.6	9.6	—	—	—	—	—	—	83.1
8	3	231.4	30.9	103.1	1.7	—	—	—	—	—	—	36.7
9	6	—	—	—	—	25.7	6.7	—	—	—	18.5	5.1
10	6	—	—	—	—	63.2	5.2	—	—	2.2	14.8	8.5
11	12	—	—	—	—	128.8	54.0	31.6	28.7	21.0	11.5	26.6
12	12	—	—	—	—	126.5	51.5	28.7	23.4	18.8	8.8	25.8
13	24	—	—	—	—	8.9	5.8	—	—	8.5	22.6	4.6
14	24	—	—	—	—	10.2	3.8	—	—	13.7	28.0	5.6

Parenteral administration. Two vitamin A-deficient rats were given 5,6-monoepoxy- β -carotene (0.05mg./rat/day) intraperitoneally for 2 days. The animals were killed 24hr. after the last dose. The extracts from the liver, intestine, kidney and spleen showed the characteristic absorption spectra and other chemical reactions of 5,6-monoepoxy- β -carotene. The total recovery of 5,6-monoepoxy- β -carotene from all the tissues examined was only 4% of the administered 5,6-monoepoxy- β -carotene. No trace of either β -carotene or 5,8-monoepoxy- β -carotene was detected in any of the tissues. 5,6-Monoepoxy- β -carotene, when administered at a dose of 1.0mg., also failed to give rise to any vitamin A or epoxyvitamin A compounds. Administration of 5,6:5',6'-diepoxy- β -carotene gave no trace of vitamin A, 5,6-monoepoxyvitamin A or β -carotene.

Biological potency of crystalline 5,6-monoepoxy- β -carotene. The growth rate (g./week) of individual litter-mate rats receiving crystalline 5,6-monoepoxy- β -carotene or all-*trans*- β -carotene was measured. The mean weekly gain in weight plotted against the logarithm of the dose gave lines for the standard and test doses that were parallel to each other. Doses of 2.5 and 5.0 μ g. of either 5,6-monoepoxy- β -carotene or β -carotene to rats seem to be within the critical range of bioassay. The biopotency of 5,6-monoepoxy- β -carotene as compared with that of all-*trans*- β -carotene, calculated from these results, was 21.4%.

DISCUSSION

5,6-Monoepoxy- β -carotene, unlike 5,6-monoepoxyretinal, gives rise to vitamin A in rats when administered orally (Table 1). Time-course studies on its absorption indicate that the small intestine is the major site for its conversion into vitamin A (Table 2), and intraperitoneal administration, enterectomy and intrainstestinal experiments support the view that the small intestine is the site of conversion of this compound into vitamin A. The extent of conversion of this compound into vitamin A is considerably lower than that of β -carotene (Glover, Goodwin & Morton, 1948). The biological potency of 5,6-monoepoxy- β -carotene is 21% of that of β -carotene. This can be explained on the basis of the observation that at least 12.8% of the administered 5,6-monoepoxy- β -carotene is converted, during its passage through the acidic conditions of the stomach, into its furanoid form (mutatochrome), which can no longer serve as vitamin A precursor.

Two mechanisms can be proposed for the conversion of 5,6-monoepoxy- β -carotene into vitamin A. First, the compound might undergo de-epoxidation in the body and be subsequently metabolized as

β -carotene; but, since 5,6-monoepoxyretinal is not de-epoxidized in the rat (Lakshmanan *et al.* 1965), this is not likely. In addition, no traces of β -carotene were detected anywhere in the body after the oral administration of 5,6 - monoepoxy - β - carotene. Secondly, 5,6-monoepoxy- β -carotene might be oxidatively cleaved at the oxygenated β -ionone ring, subsequent β -oxidation giving rise to vitamin A. The possibility of oxidative cleavage at the other β -ionone ring moiety is remote because, in this case, 5,6-monoepoxyvitamin A would be formed, as originally proposed.

In the light of the present findings, the second mechanism seems to be the most feasible one, and it is also in accordance with the mechanism of cleavage of β -carotene to vitamin A proposed by Glover & Redfearn (1954) by fission at one of the two β -ionone rings and not at the centre of the chain.

In contrast with 5,6-monoepoxy- β -carotene, 5,6:5',6'-diepoxy- β -carotene gives rise neither to vitamin A nor to 5,6-monoepoxyvitamin A in the rat. It also fails to give rise to β -carotene; hence it does not undergo de-epoxidation in the body. This compound is partially converted into its furanoid compound in the stomach. Since the compound gave no traces of 5,6-monoepoxyvitamin A, it is very likely that the epoxy groups in the β -ionone rings facilitate the cleavage of both rings and consequent destruction of the whole molecule by β -oxidation, thus explaining the negligible biological activity observed when the compound is given to vitamin A-deficient rats.

It is very likely that 5,6-monoepoxyvitamin A compounds have to be derived only from vitamin A if the vitamin is to exert its biological functions through these epoxides.

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