Studies on the Relative Biopotencies and Intestinal Absorption of Different Apo- β -Carotenoids in Rats and Chickens

By RAM VIR SHARMA, SATYA N. MATHUR* and JAGANNATH GANGULY
Department of Biochemistry, Indian Institute of Science, Bangalore-560 012, India

(Received 12 April 1976)

1. The biopotencies relative to β -carotene of several apocarotenoids, such as 8'-, 10'- and 12'-apo- β -carotenol and methyl 8'-apo- β -carotenoate, were investigated in rats, on a molar basis, by both curative-growth assay and liver-storage tests. 2. In the curative-growth assays, on a molar basis the biopotencies of 8'-, 10'- and 12'-apo- β -carotenol and methyl 8'-apo- β -carotenoate were 72, 78, 72 and 53% respectively, whereas on a weight basis the corresponding values were 93, 111, 111 and 63%, with respect to β -carotene taken as 100%. In terms of yield of vitamin A, these values were much lower in the liver-storage tests. 3. When 8'-apo- β -carotenol was fed, the unchanged aldehyde together with small amounts of the corresponding alcohol and larger proportions of the acid rapidly appeared in the tissues of both rats and chickens. The 8'-apocarotenol, 8'-apocarotenoic acid and its methyl ester were absorbed unchanged. The free acid disappeared most rapidly from the tissues, but its methyl ester persisted in the tissues longest. 4. On the basis of these observations it is suggested that most of an apocarotenol is oxidized to the corresponding acid, which, in turn, is mostly degraded to retinoic acid, with small proportions of it being attacked by the dioxygenase system giving retinal.

Synthetic apocarotenoids are being extensively used for colouring egg yolk for consumer acceptance. No doubt there has been some work on the relative biopotencies of some of the synthetic apocarotenoids, but the results reported by different groups of investigators have not been consistent. Thus Marusich *et al.* (1959) had stated that 12'-apo- β -carotenol was 125% as active as β -carotene, but according to Marusich *et al.* (1960) 8'-apo- β -carotenol was 72.8% as active. On the other hand, Glover (1959) had claimed that the biopotencies of 8'-, 10'- and 12'-apo- β -carotenol relative to β -carotene were 40, 75 and 125% respectively. The values reported by Isler *et al.* (1965) were 47.7, 53.3 and 76.4% for 4'-, 8'- and 12'-apo- β -carotenol respectively, and, according to Hoppe *et al.* (1971), 8'-apo- β -carotenoic acid ester is 78% as active as β -carotene. Values reported by Singh *et al.* (1972) are 31.0, 76.0 and 44.0% for 8'-, 10'- and 12'-apo- β -carotenol respectively, relative to β -carotene.

The weights of different apocarotenoids vary, depending on the number of carbon atoms they contain. But all these earlier investigations were carried out on a weight basis. Therefore it appeared to us that a comparison of the biopotencies of various apocarotenoids on a molar basis might give more meaningful results. In the present work, attempts were made to compare the relative biopotencies of

several synthetic apocarotenoids on a molar basis by both curative-growth and liver-storage tests, and it is shown here that on a molar basis the apocarotenoids show almost comparable growth-promoting activities, whereas the liver-storage tests give variable values.

Materials and Methods

Male rats of this Institute strain and White Leghorn chicks obtained from the State Poultry Farm at Hesarghatta, Bangalore, India, were used throughout this study. Further treatments of the animals are described in the respective places.

Silica gel C was from Acme Synthetic Chemicals, Bombay, India; it contains 13% (w/w) $\text{CaSO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$ as binder. Neutral alumina, obtained from M. Woelm (Eschwege, Germany), was deactivated with water (10%, w/v) before use for chromatography. Sources and methods of purification of all solvents and reagents were as described earlier (Mahadevan *et al.*, 1959, 1961, 1963; Ganguly *et al.*, 1959).

Synthetic all-*trans*- β -carotene and the apocarotenoids (except 8'-apo- β -carotenol and the free apocarotenoic acids) were supplied by Hoffmann-La Roche (Basle, Switzerland) and BASF, Ludwigshafen, West Germany. They were purified by chromatography through deactivated alumina columns. The absorption spectra of the purified apocarotenoids agreed well with those described in the

*Present address: Department of Biochemistry, College of Medicine, The University of Iowa, Iowa City, IA 52240, U.S.A.

literature (Schwieter & Isler, 1967; Vetter *et al.*, 1971).

8'-Apo- β -carotenol was prepared by NaBH₄ reduction of 8'-apo- β -carotenal. After repeated chromatography through deactivated alumina columns it was crystallized with a final yield of about 90%. Its spectrum in light petroleum (b.p. 40–60°C) was identical with that reported by Vetter *et al.* (1971), and its molar extinction value was 120000 at 425 nm in light petroleum. Its R_F value on silica-gel C plates was 0.2 [solvent system: acetone/light petroleum (1:10, v/v)] and 0.55 during reverse-phase ring-chromatography on Whatman no. 1 filter paper impregnated with 5% silicone fluid [solvent system: methanol/butanol/water (8:1:1, by vol.)].

The methyl esters of the apocarotenoid acids were prepared from the corresponding apocarotenals by the method of Barua *et al.* (1969). The esters thus obtained were crystallized after alumina chromatography with a yield of about 70%. The R_F values on t.l.c. and the spectral properties of the free acids, obtained by hydrolysis of the methyl esters with methanolic 10% (w/v) KOH, closely agreed with those reported in the literature (Schwieter & Isler, 1967; Singh *et al.*, 1973).

Retinal was prepared from crystalline retinol as described by Ball *et al.* (1948), and was purified by chromatography through deactivated alumina columns.

Analysis, identification and determination of the carotenoids

In all experiments, the carotenoids were extracted as described by Thompson *et al.* (1950), after which the extracts in light petroleum were chromatographed through columns of water-deactivated alumina (10%, v/w) and the compounds were successively eluted with the following eluents: light petroleum, β -carotene and retinyl ester; 1% (v/v) benzene in light petroleum, apocarotenyl esters and methyl apocarotenoate; 2% (v/v) acetone in light petroleum, retinal and apocarotenals; 6% (v/v) acetone in light petroleum, retinol and apocarotenols; 30% (v/v) acetic acid in light petroleum, apocarotenoid acids and retinoic acid. The respective fractions were further purified by t.l.c. on silica-gel C plates by using the following solvent systems: 6% (v/v) acetone in light petroleum for the apocarotenals and methyl apocarotenoate, 10% (v/v) acetone in light petroleum for the apocarotenals and apocarotenols and 25% (v/v) acetone in light petroleum for the apocarotenoid acids. After t.l.c. they were identified from their R_F values during reverse-phase ring chromatography on silicone-impregnated paper (5% silicone fluid) with the solvent system methanol/water (9:1, v/v) for all compounds; their R_F values closely agreed with those

of the standard samples. They were further identified from their spectral properties in different solvent systems, such as light petroleum, ethanol and chloroform, and their spectra closely agreed with those of the reference samples. Their concentrations were calculated from the extinction values at the $\lambda_{\max}$, against the values reported in the literature.

When 8'-apo- β -carotenol was fed, along with the unchanged apocarotenol, another fraction of apocarotenoid was isolated from the tissues. On saponification with methanolic KOH this fraction yielded the parent apocarotenol, which was identified as described above. It is therefore possible that it is a fatty acid ester of the apocarotenol.

Usually it was rather difficult quantitatively to elute the apocarotenoid acids from the alumina columns because of some loss of these compounds. In such cases, after extraction from the tissues the light petroleum was evaporated and the residue was dissolved in ethanol and made alkaline with dilute NaOH; the aldehydes and alcohols (if any) were extracted with light petroleum, after which the apocarotenoid acids were extracted with the same solvent after acidification of the aqueous phase with acetic acid. The apocarotenoid acids thus isolated were subsequently identified against reference compounds which were prepared as described above.

Retinol and its esters were estimated by the Carr-Price reaction.

Results

Relative pro-vitamin A activities of the apocarotenoids

Curative growth assay. (i) In rats. In these trials the U.S.P.XIII procedure, as adopted by Deuel *et al.* (1953), was followed. Weanling male rats were raised on the vitamin A-deficient diet as described by Malathi *et al.* (1963) and they were considered deficient when they did not gain in weight for 4 consecutive days. Usually they reached the weight-plateau stage within 4 weeks after they were first put on the deficient diet and at this stage they were given supplements of the carotenoids, as described in the legend to Fig. 1, after which they were weighed daily for 28 days. Fig. 1 shows that, when plotted against log dose, the weight gains gave virtual straight lines and that all lines are almost parallel to that obtained with the standard β -carotene, which validated the experiments. These results are further summarized in Table 1, which shows that the biopotencies were 72, 78, 72 and 53% for the 8', 10' and 12'-apo- β -carotenol and methyl 8'-apo- β -carotenoate respectively, against β -carotene taken as 100%, on a molar basis; on a weight basis the respective values were 93, 111, 111 and 63%, against β -carotene as standard.

(ii) In chickens. In another set of experiments attempts were made to use chickens for determining

the relative biopotencies of the apocarotenoids by the procedure of Zechmeister *et al.* (1952), and here 300 1-day-old White Leghorn male chicks were

Fig. 1. Growth response of the different apocarotenoids in rats relative to β -carotene as standard

The carotenoids were given daily in 0.1 ml of groundnut oil containing 0.5% α -tocopherol; the negative controls received 0.1 ml of groundnut oil containing 0.5% α -tocopherol. $\blacktriangle$, β -Carotene; $\circ$, 8'-apo- β -carotenal; $\triangle$, 10'-apo- β -carotenal; $\square$, 12'-apo- β -carotenal; $\bullet$, methyl 8'-apo- β -carotenoate.

maintained on the vitamin A-deficient diet of Joshi *et al.* (1973). They became deficient within 15–20 days. At this stage they were divided into several groups and were given, in a similar fashion, daily supplements of 1, 2 or 3 nmol of β -carotene or 2, 4 or 6 nmol of the different apocarotenoids. Since the rates of their growth were much higher than in rats, the lower doses of the carotenoids were not sufficient to sustain their growth, as a consequence of which there was a very high rate of mortality in the groups receiving 2 and 4 nmol of the apocarotenoids or 1 and 2 nmol of β -carotene. On the other hand, the birds receiving 6 nmol of the apocarotenoids or 3 nmol of β -carotene grew well. But, here, their weight gain was much too high after 18 days. Therefore the weight gain on the 18th day only was taken and the relative weight gains, calculated at the levels of 6 nmol of the apocarotenoids against 3 nmol of β -carotene, were 32, 50, 55 and 52% for 8', 10', 12'-apo- β -carotenal and methyl 8'-apo- β -carotenoate respectively, against β -carotene taken as 100%. Obviously those values do not strictly reflect the exact biopotencies, because they could be calculated at one dosage only and only on the 18th day of supplementation.

Liver-storage test. In these experiments most of the initial reserves of vitamin A were depleted by keeping weanling male rats on the vitamin A-deficient diet of Malathi *et al.* (1963) for 3 weeks, after which they were given the carotenoids as indicated in Table 2. The

Table 1. Curative-growth assay of the apocarotenoids in rats

Summary of the total gain in weight of vitamin A-deficient rats receiving β -carotene or the apocarotenoids. The carotenoids were given daily in 0.1 ml of groundnut oil containing 0.5% α -tocopherol; the negative controls received 0.1 ml of groundnut oil containing 0.5% α -tocopherol. The values are calculated from Fig. 1. S.D. values are given for the mean gain in weight and for the % biopotency calculated on a molar basis. The values given in parentheses are the numbers of rats that survived.

Supplement	Dose/day per rat (nmol)	No. of rats used	Depletion period		Assay period (28 days)			Biopotency compared with β -carotene on a molar basis (%)
			Mean duration (days)	Mean final wt. (g)	Mean low wt. (g)	Mean final wt. (g)	Mean gain in wt. (g)	
Negative control	0	14	28.2	71.8	71.8	64.3	-7.5 ± 2.8 (2)	
β -Carotene	1	10	28.1	80.6	80.2	126.6	46.4 ± 5.8 (10)	
	2	10	28.0	81.0	80.7	152.8	72.1 ± 6.3 (10)	100
	3	10	28.0	67.2	67.2	155.5	88.3 ± 6.4 (10)	(100)*
8'-Apo- β -carotenal	2	10	28.2	81.4	79.3	131.4	52.1 ± 7.1 (10)	
	4	10	28.0	77.5	74.9	154.7	79.8 ± 6.2 (10)	72 ± 3.7
	6	10	28.4	84.0	81.7	165.9	84.2 ± 5.2 (10)	(93)*
10'-Apo- β -carotenal	2	10	28.3	85.4	82.8	138.9	56.1 ± 6.8 (10)	
	4	10	28.0	77.6	74.5	157.7	83.2 ± 5.1 (10)	78 ± 2.8
	6	10	28.4	67.6	66.2	154.8	88.6 ± 4.8 (10)	(111)*
12'-Apo- β -carotenal	2	10	28.3	68.8	68.8	120.9	52.1 ± 8.1 (10)	
	4	10	28.0	75.5	73.5	150.8	77.3 ± 6.7 (10)	72 ± 5.1
	6	10	28.0	75.5	74.0	157.2	83.2 ± 4.3 (10)	(111)*
Methyl 8'-apo- β -carotenoate	2	10	28.0	79.0	73.2	111.6	38.4 ± 6.7 (8)	
	4	10	28.0	83.3	79.8	144.1	64.3 ± 6.5 (10)	53 ± 4.8
	6	10	28.0	75.3	71.1	151.4	80.3 ± 8.6 (10)	(63)*

* % biopotency calculated on a weight basis. (Confidence limit more than 95%.)

Table 2. *Liver-storage tests of the apocarotenoids in rats*

Summary of relative yields of vitamin A in the livers of rats receiving β -carotene or different apocarotenoids. The rats were given in 1 week, in three equal doses, the indicated amounts of the carotenoids dissolved in 0.5 ml of groundnut oil containing 0.5% α -tocopherol. The negative controls received groundnut oil containing α -tocopherol. The average biopotencies are calculated on a molar basis, and the values in parentheses indicate the corresponding values when calculated on a weight basis. Results are means $\pm$ s.d.

Supplement	Dose/week per rat (μ mol)	No. of rats used	Total vitamin A/liver (nmol)	Apocarotenoid/liver (nmol)	Relative amounts of vitamin A in the liver (%)	
Negative control	0	6	—	—		
β -Carotene	1	6	20 $\pm$ 3.2	—	100	
	2	6	60 $\pm$ 5.1	—		
	3	6	98 $\pm$ 6.3	—		
8'-Apo- β -carotenol	2	6	16 $\pm$ 2.9	—	45.0	45.6 (58.2)
	4	6	55 $\pm$ 5.5	—	45.8	
	6	6	90 $\pm$ 7.2	—	45.9	
8'-Apo- β -carotenal	2	6	8 $\pm$ 1.4	—	20.0	20.4 (26.2)
	4	6	25 $\pm$ 2.4	—	20.8	
	6	6	40 $\pm$ 3.6	—	20.4	
8'-Apo- β -carotenoic acid	2	6	5 $\pm$ 0.9	—	15.0	14.7 (18.2)
	4	6	18 $\pm$ 1.2	—	15.0	
	6	6	28 $\pm$ 3.1	—	14.2	
Methyl 8'-apo- β -carotenoate	2	6	3 $\pm$ 0.7	270 $\pm$ 23	7.5	7.5 (9.0)
	4	6	10 $\pm$ 1.4	525 $\pm$ 37	8.3	
	6	6	13 $\pm$ 1.8	650 $\pm$ 32	6.6	

animals were killed 72 h after the last dose, and the livers were removed and analysed. No significant amounts of the carotenoids were found in the livers, except in the rats receiving methyl apocarotenoate. This particular compound was well absorbed and was present in large amounts in the liver, as shown in Table 2 (see also Fig. 2). In these cases, the liver extracts were chromatographed as described in the Materials and Methods section, after which the retinol and retinyl ester fractions were combined and measured as total vitamin A by the Carr-Price reaction, and the carotenoids were determined spectrophotometrically.

Table 2 shows that in terms of the actual amounts of vitamin A deposited in the liver of rats receiving comparable amounts of the apocarotenoids, the respective values for the 8'-apo- β -carotenol, 8'-apocarotenol, 8'-apocarotenoic acid and methyl 8'-apocarotenoate were 45.6, 20.4, 14.7 and 7.5% relative to β -carotene taken as 100%, on a molar basis. On the other hand, when calculated on a weight basis, the respective values were 58.2, 26.2, 18.2 and 9.0%.

Relative rates of appearance of the different apocarotenoids in the tissues of rats after feeding of these compounds

Here also weanling male rats were depleted of their reserves of vitamin A by keeping them on the vitamin

A-deficient diet for 3 weeks. Fig. 2 shows that all the compounds could be detected in considerable amounts in the intestinal contents and mucosa up to 8 h after the dose, after which they disappeared rather rapidly. But, although the free acid and its methyl ester did not give rise to any detectable amounts of any other metabolites in the samples analysed, the aldehyde yielded the corresponding acid and alcohol ester (probably esterified with an endogenous fatty acid), in both the intestinal contents and mucosa. The apocarotenol on the other hand gave rise to its ester and no other metabolite.

The amounts of the carotenoids in blood were rather small and therefore they were determined as total carotenoids. The peak of appearance in blood was at 2–3 h for 8'-apo- β -carotenol, 1.5–2 h for 8'-apo- β -carotenoic acid and 3–4 h for 8'-apo- β -carotenol and methyl 8'-apo- β -carotenoate. It should, however, be noted that, whereas the concentration in blood of the free acid was very low, that of its ester was highest of all the carotenoids tested.

Such marked differences in the appearance of the carotenoids in blood were reflected in the livers also. Thus the methyl ester was present in largest concentrations in the liver, and it persisted there until the termination of the experiment with slight changes in its net amounts; no other metabolites derived from it could be detected there. The amounts of the free acid were very low in the liver and it virtually disappeared after 6 h. After feeding of the aldehyde,

Fig. 2. Appearance of the different apocarotenoids and their metabolites in the tissues of rats after oral administration

After starvation for 24h, vitamin A-depleted rats were given, in one single dose and with the aid of a stomach tube, 10 μ mol of each of the apocarotenoids, dissolved in 0.5 ml of groundnut oil containing 0.5% α -tocopherol, except that the apocarotenoid acid was given as its sodium salt in 0.5 ml of buffer (0.1 M-phosphate, pH 7.4). The animals were killed at the indicated time-intervals after the dose by direct heart puncture while under light ether anaesthesia. Removal of the tissues, separation of the intestinal contents and mucosa, and extraction of the samples were as described by Murthy *et al.* (1963) and Deshmukh & Ganguly (1967), and purification and determination of the apocarotenoids were as described in the Materials and Methods section. Extracts from the tissues of three animals were pooled within a group, and the values given here are means of two such groups for each time-interval and are expressed as nmol of the apocarotenoid/10 ml of blood or whole organ. The apocarotenoids appearing in the tissues are indicated by the following symbols: ■, 8'-apo- β -carotenol; □, 8'-apo- β -carotenol ester; ○, 8'-apo- β -carotenol; ●, methyl 8'-apo- β -carotenoate. The apocarotenoids administered to the rats are indicated at the top of each set of curves. (a) Apocarotenoids in the intestinal contents; (b) those in intestinal mucosa; (c) those in the blood; (d) those in the liver.

appreciable amounts of the corresponding acid and small amounts of the alcohol were found in the liver. Administration of the alcohol on the other hand led to the appearance of the parent alcohol together with considerable amounts of its ester; the concentrations of both components started to decline after 8 h and they virtually disappeared after 48 h. But here no corresponding aldehyde or acid could be found.

Attempts were made to detect vitamin A in the intestine 4 h after feeding with the apocarotenoids, when 6–7 nmol of retinol and its esters were found in the rats given the apocarotenol, with the apocarotenol yielding 9–10 nmol; no vitamin A could be detected in the same tissue of the rats receiving the other two apocarotenoids.

Experiments with chickens

In similar experiments with vitamin A-depleted chicks these apocarotenoids were similarly well absorbed, but they disappeared from their intestine much faster and virtually none of the apocarotenoids could be detected in the chick intestine 8 h after the dose, which was reflected in the other tissues also. One of the notable aspects of these studies was that no striking species difference could be noticed in the absorption of the apocarotenoids, as is usually found with the common C₄₀ carotenoids in that, whereas rats do not absorb any of the common C₄₀ carotenoids, chickens absorb the hydroxy C₄₀ carotenoids. Therefore it appears that during absorption, the apocarotenoids behave more like retinol and its derivatives in both species of animals.

Discussion

It is rather difficult to interpret ~~the~~ results against the background of our existing knowledge of metabolism of carotenoids. Two theories have been proposed for the mechanism of conversion of β -carotene into vitamin A. According to one of them, the β -carotene molecule is eccentrically cleaved, yielding apocarotenals, which in turn are stepwise degraded by a mechanism analogous to β -oxidation, ultimately giving retinol. According to the second theory the carotene is specifically cleaved at the 15–15' double bond by a specific dioxygenase system, giving two molecules of retinal, which in turn are reduced to retinol. On the basis of evidence of chemical oxidation of β -carotene, Glover (1960) has discussed the possibilities of the first theory. But, since then, Mize *et al.* (1966) have demonstrated that isoprenoid compounds (e.g. phytanic acid and pristanic acid) are metabolized through pathways analogous to β -oxidation, which would mean that the apocarotenoids should give retinoic acid rather than retinol.

If, on the other hand, the degradation were to take place via the dioxygenase system (Goodman *et al.*,

1966; Olson, 1969), on a molar basis all apocarotenoids should have given comparable amounts of vitamin A in the liver and at the same time should have shown comparable growth response, but this was not so. Thus in the curative-growth assays only the methyl 8'-apocarotenoate was about one-half as active as β -carotene, and all other apocarotenoids were about 70–80% as active, on a molar basis. In the liver-storage tests, on the other hand, although the apocarotenol gave about one-half as much vitamin A as did β -carotene, all other apocarotenoids gave much lower amounts, with the methyl apocarotenoate giving the lowest amounts.

It has also been shown here that the various apocarotenoids differ markedly in storage, metabolic transformation and survival in the tissues of both rats and chickens. Thus the apocarotenoic acid was poorly stored, and it disappeared most rapidly from the tissues. The corresponding aldehyde, on the other hand, persisted in the tissues for a longer time, but at the same time it was both oxidized and reduced to the corresponding acid and alcohol. The behaviour of the apocarotenol was similar to that of the aldehyde, except that its oxidation products could not be detected in the tissues and that it was esterified to an appreciable extent with endogenous fatty acids. In contrast, considerable amounts of the methyl apocarotenoate were present in the liver till the termination of the experiments; no free acids could be found after feeding this compound.

We have noticed (R. V. Sharma, S. N. Mathur, A. A. Dmitrovskii, R. C. Das & J. Ganguly, unpublished work) that the particulate fractions of the homogenates of rat and chicken liver can rapidly oxidize the apocarotenals to the corresponding acids in the presence of NAD⁺ or NADP⁺. It is therefore possible that the bulk of the apocarotenol is rapidly oxidized to the corresponding apocarotenoic acid, which is then degraded to the lower acids, and that small proportions of the apocarotenol are reduced to the corresponding alcohol. It is also possible that, if the enzyme that hydrolyses the methyl apocarotenoate is very weakly active, only small amounts of the apocarotenoic acid are released for degradation. The apocarotenol on the other hand is probably slowly oxidized to the corresponding apocarotenal by the rather non-specific alcohol dehydrogenase; the apocarotenal thus formed would then be rapidly oxidized to the corresponding apocarotenoic acid and then to the lower acids. There is some evidence in support of such an interpretation, because we have isolated and identified lower apocarotenoic acids, such as the 10'- and 12'- acids, from the intestine of rats fed on 8'-apo- β -carotenol (R. V. Sharma, S. N. Mathur, A. A. Dmitrovskii, R. C. Das & J. Ganguly, unpublished work).

Not only have these apocarotenoids shown growth response in the curative-growth tests, but

they have also yielded some vitamin A in the liver-storage tests. It is therefore clear that some parts of the apocarotenoids are simultaneously attacked by the dioxygenase system also. After feeding of β -carotene, we (R. V. Sharma, S. N. Mathur, A. A. Dmitrovskii, R. C. Das & J. Ganguly, unpublished work) have been able to identify several apocarotenals in chicken intestine, together with higher proportions of retinal. Therefore it is possible that the β -carotene molecule may be simultaneously attacked at several double bonds by the dioxygenase (or different dioxygenases), giving retinal and other apocarotenals, the major attack being at the central double bond. Whereas retinal can give retinoic acid and retinol, the apocarotenals would give mostly retinoic acid with small amounts of retinol. In such a situation, retinol would account for both growth and liver storage, whereas the retinoic acid should account for growth only, because it can neither be reduced to retinol nor can it be stored.

Yet another point that should receive serious consideration in this context is the question of relative biopotencies of β -carotene and vitamin A. If the dioxygenase were to attack the β -carotene molecule specifically at the 15-15' double bond, as has been claimed in many reports (Olson, 1961, 1969; Olson & Hayaishi, 1965; Goodman *et al.*, 1966; Lakshmanan *et al.*, 1968, 1972; Fidge *et al.*, 1969; Singh & Cama, 1974), on a weight basis, β -carotene should be as active as vitamin A. But, except for a few isolated reports (KoeHN, 1948; Burns *et al.*, 1951), it has been universally accepted that on a weight basis β -carotene is about 50-60% as active as vitamin A. This phenomenon can be explained only on the basis of excentric cleavage of the carotene molecule.

This work was supported by a U.S. PL-480 research grant no. A7-AH-84. We thank Hoffmann-La Roche, Basle, Switzerland, and BASF, Ludwigshafen, West Germany, for generous gifts of synthetic apo- β -carotenoids and β -carotene used in these experiments.

References

- Ball, S., Goodwin, T. W. & Morton, R. A. (1948) *Biochem. J.* **42**, 516-523
- Barua, A. B., Ghosh, M. C. & Goswami, K. (1969) *Biochem. J.* **113**, 447
- Burns, M. J., Hauge, S. M. & Quackenbush, F. W. (1951) *Arch. Biochem.* **30**, 341-346
- Deshmukh, D. S. & Ganguly, J. (1967) *Indian J. Biochem.* **4**, 18-21
- Deuel, H. J., Jr., Ganguly, J., Wallcave, L. & Zechmeister, L. (1953) *Arch. Biochem. Biophys.* **47**, 237-240
- Fidge, N. H., Smith, F. R. & Goodman, D. S. (1969) *Biochem. J.* **114**, 689-694
- Ganguly, J., Krishnamurthy, S. & Mahadevan, S. (1959) *Biochem. J.* **71**, 756-762
- Glover, J. (1959) *Annu. Rep. Prog. Chem.* **56**, 331-342
- Glover, J. (1960) *Vitam. Horm. (N.Y.)* **18**, 371-386
- Goodman, D. S., Huang, H. S. & Shiratori, T. (1966) *J. Biol. Chem.* **241**, 1929-1932
- Hoppe, P., Gropp, J. & Tiews, J. (1971) *Arch. Geflügelkd.* **35**, 75-76
- Isler, O., Lindlar, H., Montavon, M., Rüegg, R., Saucy, G. & Schwieter, U. (1965) cited in *Chem. Abstr.* (1965) **63**, 17045d
- Joshi, P. S., Mathur, S. N., Murthy, S. K. & Ganguly, J. (1973) *Biochem. J.* **136**, 757-761
- KoeHN, C. J. (1948) *Arch. Biochem.* **17**, 337-344
- Lakshmanan, M. R., Pope, J. L. & Olson, J. A. (1968) *Biochem. Biophys. Res. Commun.* **33**, 347-352
- Lakshmanan, M. R., Chansang, H. & Olson, J. A. (1972) *J. Lipid Res.* **13**, 477-482
- Mahadevan, S., Krishnamurthy, S. & Ganguly, J. (1959) *Arch. Biochem. Biophys.* **83**, 371-375
- Mahadevan, S., Murthy, S. K., Krishnamurthy, S. & Ganguly, J. (1961) *Biochem. J.* **79**, 416-424
- Mahadevan, S., Seshadri Sastry, P. & Ganguly, J. (1963) *Biochem. J.* **88**, 531-534
- Malathi, P., Seshadri Sastry, P., Subba Rao, K. & Ganguly, J. (1963) *Biochem. J.* **87**, 305-311
- Marusich, W., De Ritter, E., Vreeland, J. & Krukar, R. (1959) *Am. Chem. Soc. Meet., Sept. 1959* [quoted in Glover (1960) *Vitam. Horm. (N.Y.)* **18**, 371-386]
- Marusich, W., De Ritter, E., Vreeland, J. & Krukar, R. (1960) *J. Agric. Food Chem.* **8**, 390-393
- Mize, C. E., Steinberg, D., Avigan, J. & Fales, H. M. (1966) *Biochem. Biophys. Res. Commun.* **25**, 359-365
- Murthy, S. K., David, J. S. K. & Ganguly, J. (1963) *Biochim. Biophys. Acta* **70**, 490-492
- Olson, J. A. (1961) *J. Biol. Chem.* **236**, 349-356
- Olson, J. A. (1969) *Am. J. Clin. Nutr.* **22**, 953-962
- Olson, J. A. & Hayaishi, O. (1965) *Proc. Natl. Acad. Sci. U.S.A.* **51**, 1364-1370
- Schwieter, U. & Isler, O. (1967) in *The Vitamins* (Sebrell, W. H., Jr. & Harris, R. S., eds.), vol. 1, pp. 5-101, Academic Press, London and New York
- Singh, H. & Cama, H. R. (1974) *Biochim. Biophys. Acta* **370**, 49-61
- Singh, H., Krishna Mallia, A. & Cama, H. R. (1972) *Biochem. Soc. Symp.* **35**, 219-232
- Singh, H., Jacob, J. & Cama, H. R. (1973) *J. Chromatogr.* **75**, 146-150
- Thompson, S. Y., Braude, R., Coates, M. E., Cowie, A. T., Ganguly, J. & Kon, S. K. (1950) *Br. J. Nutr.* **4**, 398-421
- Vetter, W., Euglert, G., Rigassi, N. & Schwieter, U. (1971) in *Carotenoids* (Isler, O., ed.), pp. 189-266, Birkhauser Verlag, Basel and Stuttgart
- Zechmeister, L., Deuel, H. J., Jr., Inhoffen, H. H., Leemann, J., Greenberg, S. M. & Ganguly, J. (1952) *Arch. Biochem. Biophys.* **36**, 80-88