Synthesis and metabolism of all-*trans*-[11-³H]retinyl β -glucuronide in rats *in vivo*

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All-*trans*-[11-³H]retinyl β -glucuronide (all-*trans*-[11-³H]ROG) was synthesized from [³H]retinol by an improved synthetic procedure. After its intraperitoneal injection into rats, ROG is initially found as the predominant labelled component in the serum, but then is distributed to the liver, intestine, kidney and other organs of the body. Esters of vitamin A, which constituted the major metabolite of ROG, were detected in the liver as well as in other tissues. Of the labelled vitamin A esters derived from tritiated ROG in the liver and intestine, about 50% contained 5,6-epoxyretinol, which was characterized by its chromatographic behaviour, formation of an acetyl ester and lack of reactivity with diazomethane. Thus ROG, although converted to retinol *in vivo*, might also act physiologically in an intact form.

INTRODUCTION

Retinoyl and retinyl β -glucuronides, first characterized in the bile of rats treated with retinoic acid or with retinol (Dunagin *et al.*, 1966; Zachman *et al.*, 1966; Lippel & Olson, 1968), are highly active in promoting the growth of rats (Nath & Olson, 1967; Barua & Olson, 1985, 1987) and the cellular differentiation both of vaginal epithelia (Sietsema & DeLuca, 1982) and of HL-60 promyelocytic leukaemia cells (Gallup *et al.*, 1987; Zile *et al.*, 1987). While being less cytotoxic than other retinoids towards HL-60 cells (Gallup *et al.*, 1987; Zile *et al.*, 1987), the retinoid β -glucuronides also are highly active in inhibiting the induction of neoplastic changes in mammary gland cultures (Mehta *et al.*, 1987). In addition to their formation in the liver (Dunagin *et al.*, 1966; Lippel & Olson, 1968; Sklan & Halevy, 1984), retinoid β -glucuronides are synthesized in the intestine *in vivo* (Zile *et al.*, 1982; Cullum & Zile, 1985) and *in vitro* (Miller & DeLuca, 1986). Furthermore, both retinoid β -glucuronides are endogenous components of human plasma (Barua & Olson, 1986; Barua *et al.*, 1986).

Both all-*trans*-retinoyl β -glucuronide (Barua & Olson, 1985) and all-*trans*-retinyl β -glucuronide (all-*trans*-ROG) (Barua & Olson, 1986, 1987) have recently been synthesized in good yield and have been well characterized by physicochemical methods.

In the present study, we have synthesized retinyl β -glucuronide labelled with [³H]retinol by an improved procedure and have investigated its metabolism in rats *in vivo*.

MATERIALS AND METHODS

Chemicals and solvents

Products and their purveyors were: all-*trans*-retinyl acetate, all-*trans*-retinoic acid and β -glucuronidase from *Escherichia coli* (activity 570 000 units/g) (Sigma Chemical Co., St. Louis, MO, U.S.A.); [11-³H]retinoic acid (SRI International, Menlo Park, CA, U.S.A.), through the courtesy of the National Cancer Institute, Bethesda,

MD, U.S.A.); silver carbonate and Diazald (*N*-methyl-*N*-nitroso-*P*-toluenesulphonamide) (Aldrich Chemical Co., Milwaukee, WI, U.S.A.); and Biofluor for scintillation counting (New England Nuclear, Boston, MA, U.S.A.). 4-Oxoretinol and 4-hydroxyretinol were prepared from retinyl acetate (Barua & Olson, 1985), with 5,6-epoxyretinyl acetate as a by-product. The latter was hydrolysed with base to 5,6-epoxyretinol and subsequently esterified by treatment with palmitoyl chloride and other acyl chlorides. Other solvents and reagents used have been previously described (Barua & Olson, 1987).

H.p.l.c.

Retinoids were separated on a reversed-phase 'Resolve' 5 μ C₁₈ column (3.9 mm $\times$ 15 cm) (Waters Associates, Milford, MA, U.S.A.) preceded by a C-130 guard column (Upchurch Scientific, Oak Harbor, WA, U.S.A.) by using a linear gradient from CH₃OH/water (17:8, v/v) to CH₃OH/CH₂Cl₂ (4:1, v/v) at a flow rate of 1 ml/min for a 20 min period, followed by isocratic elution with the latter solvent for an additional 15 min. This procedure is similar to that reported by Furr *et al.* (1984), except that the cited solvents replaced methanol/tetrahydrofuran mixtures. A 7125 injector (Rheodyne Co., Cotati, CA, U.S.A.), 6000A and M-45 pumps with a 660 solvent programmer (Waters), a V⁴ detector (ISCO Co., Lincoln, NE, U.S.A.), and a CR-3A integrator (Shimadzu Co., Kyoto, Japan) were also used. Absorbance was monitored at 325 nm. The separation of reference retinoids in the system is depicted in Fig. 1. To restore the column, the gradient was reversed to initial conditions during 5 min, followed by a 10 min equilibration period. Synthesized [11-³H]ROG was purified on a reversed-phase ODS-2 column (0.4 cm $\times$ 30 cm) (Whatman Co., Hillsboro, OR, U.S.A.) by using 10 mM-ammonium acetate in CH₃OH/water (3:1, v/v) at a flow rate of 1 ml/min.

Synthesis of all-*trans*-[11-³H]ROG

[11-³H]Retinoic acid (8.2 $\times$ 10⁸ d.p.m. in 183 μ g), after dilution with 6.7 μ mol of unlabelled retinoic acid,

Abbreviations used: ROG, retinyl β -glucuronide; *t_R*, retention time.

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Fig. 1. H.p.l.c. profile of standard retinoids

Reversed-phase h.p.l.c. was carried out on a Waters 5 μ C₁₈ column (3.9 cm $\times$ 15 cm) by using a linear gradient for 20 min of CH₃OH/water (17:8, v/v) containing 10 mM-ammonium acetate to CH₃OH/CH₂Cl₂ (4:1, v/v) at a flow rate of 1 ml/min. Abbreviations: OROL, 4-oxoretinol; RAG, retinoyl β -glucuronide; RA, retinoic acid; EROL, 5,6-epoxyretinol; ROL, retinol; RAC, retinyl acetate; ERP, 5,6-epoxyretinyl palmitate; RL, retinyl linoleate; RP, retinyl palmitate; RS, retinyl stearate.

was converted by treatment with diazomethane to all-*trans*-methyl retinoate and then reduced with LiAlH₄ to 6.3 μ mol of retinol (yield 94%). The latter, dissolved in anhydrous diethyl ether, was treated with freshly prepared (Bollenback *et al.*, 1955) methyl (2,3,4-triacetyl-1-bromo-1-deoxy- α -D-glucopyran)uronate (8 mg), Ag₂CO₃ (16 mg), and butylated hydroxytoluene (0.2 mg) at 22 °C. After the removal of ether under argon, the oily residue was stirred with a tiny magnet for 1 h, diluted with ether (0.5 ml), and then stirred for an additional 1 h. After more ether (1 ml) was added, the mixture was warmed to 30 °C for 5 min. Insoluble material was centrifuged off and extracted three times with ether (3 $\times$ 1 ml). After these extracts were pooled with the initial ether solution, solvent was evaporated under argon, and the residue was dissolved in CH₃OH (1 ml). The ester of ROG was hydrolysed by treatment with NaOCH₃ (10 mg in 0.2 ml of CH₃OH) at 50 °C for 5 min. The solution was then neutralized with 0.1 M-acetic acid.

All-*trans*-ROG eluted from the reversed-phase ODS-2 column with a retention time (t_R value) of 10.1 min, far earlier than retinol. The ROG fraction, after dilution with water and acidification with acetic acid, was extracted three times with ethyl acetate, dried over anhydrous Na₂SO₄, evaporated under argon, and dissolved in 0.5 ml of CH₃OH containing 0.001% butylated hydroxytoluene. After measurement of its absorbancy at 325 nm, CH₃OH was removed, and ROG was dissolved in 3.5 ml of 0.85% saline solution. The final solution contained 0.76 μ mol of ROG (0.22 μ mol/ml) with a specific activity of 1.22×10^5 d.p.m./nmol. The yield was 11% in terms of the retinoic acid used. When large amounts (> 1 g) of unlabelled retinol was used in this

modified procedure, the yield was ca. 60%, 4-fold higher than that reported earlier (Barua & Olson, 1987). The major procedural change was the initial stirring of the oily residue in the absence of solvent for 1 h.

Animal studies

Male Sprague-Dawley rats ($n = 5$) weighing 180–200 g were fed a pelleted commercial diet for 2 weeks. Thereafter, [³H]ROG (108 nmol containing a total of 1.32×10^7 d.p.m. in 0.5 ml of saline) was injected intraperitoneally into each rat. Rats were anaesthetized with ether and killed 1, 3, 6, 12 or 24 h later by drawing as much blood as possible by syringe from the aorta. Blood was allowed to clot, and the serum was collected after centrifugation. At the time of killing, the small intestine, liver, kidney and other tissues were removed and washed with saline. Serum and tissues were kept frozen at –20 °C until analysis. Urine and faeces, collected for 24 h from one rat kept in a metabolic cage, were also kept frozen until analysis. Sera were analysed within 2 days, and tissues and excreta within 2 weeks.

Frozen rat sera obtained in previously reported growth studies (Barua & Olson, 1987) were also analysed. In these studies vitamin A-depleted rats received ROG (151 nmol/week/rat by intraperitoneal injection for 3 weeks. Blood was collected at the end of the third week by cutting the tip of the tail under light ether anaesthesia. The rats then received an intraperitoneal injection of ROG (714 nmol/rat) in saline. The rats were sacrificed 3.5–4.5 h after the dose and their blood was collected. The serum was frozen and stored at –20 °C.

Extraction of retinoids

(i) **Serum.** Serum (1 ml) was extracted with 2 ml each of ethanol and ethyl acetate and centrifuged. The supernatant solution was diluted with water (3 ml), acidified with 1 M-acetic acid (50 μ l), and extracted twice with ethyl acetate (2 $\times$ 2 ml). After being vortexed with water (3 ml), the mixture was centrifuged, and the ethyl acetate extract was separated, evaporated under argon, and dissolved in 100 μ l of CH₃OH/CH₂Cl₂. Portions (25–50 μ l) were injected for h.p.l.c. analysis.

(ii) **Other tissues.** Portions of frozen liver (1 g), small intestine (3 g), kidney (1 g), muscle (2 g), lung (2 g), adipose tissue (2 g) and the total faeces were each ground to a powder with two to five times their weight of anhydrous Na₂SO₄ and then were extracted first with ethyl acetate (30 ml) and then three times with CH₂Cl₂ (30 ml). The filtered extract (100–150 ml) was rotary-evaporated in a flask to 1–2 ml and the remaining solvent was removed under argon. The residue was dissolved in CH₂Cl₂ (3 ml) and transferred to a conical tube. The flask was rinsed twice with CH₂Cl₂ (1 ml), and the pooled CH₂Cl₂ solution was evaporated to dryness under argon. The residue was dissolved in 0.1–0.5 ml of CH₃OH/CH₂Cl₂ (4:1, v/v). Portions (75–200 μ l) were injected for h.p.l.c. analysis. Portions of urine (50 μ l), after dilution with an equal volume of CH₃OH, were directly analysed by h.p.l.c.

Other chemical procedures

(iii) **Saponification.** Esters in tissues (1 g) were hydrolysed by treatment with 10% KOH in CH₃OH (10 ml) for 30 min under reflux. After dilution with water (20 ml), neutral lipids were extracted three times with

Table 1. Distribution of radioactivity in serum and other tissues of rats at various times after an intraperitoneal dose of [11-³H]ROG

Rats were given 50 µg of [11-³H]ROG in saline solution by intraperitoneal injection and killed at various times. The retinoids were extracted and the radioactivity associated with each organ was determined. The volume of serum was assumed to be 4 % of body wt. Values in parentheses indicate the percentage of the total recovered radioactivity at a given time.

Time (h)	Distribution of ³ H (% of dose)			
	Serum	Liver	Small intestine	Kidney
1	13.3 (66.8)	4.3 (21.7)	1.6 (8.0)	0.7 (3.5)
3	3.4 (40.0)	3.3 (38.9)	0.6 (7.0)	1.2 (14.1)
6	2.9 (26.4)	3.8 (34.6)	3.5 (31.8)	0.8 (7.2)
12	1.7 (23.7)	3.0 (41.6)	2.1 (29.2)	0.4 (5.5)
24	2.1 (30.4)	3.4 (49.3)	1.2 (17.4)	0.2 (2.9)

hexane, and after acidification of the aqueous phase with 1 M-HCl, acidic lipids were obtained by extracting three times with ethyl acetate. After washing with water and evaporating the solvent under argon, the residue was dissolved in 0.5 ml of CH₃OH/CH₂Cl₂ (4:1, v/v). Portions (100 µl) were injected for h.p.l.c. analysis.

(iv) Acetylation. Alcohols in saponified liver extracts were acetylated by treatment with acetic anhydride in pyridine as follows: saponified liver extract (250 µl) obtained in (iii) was evaporated to dryness under argon, and the residue was dissolved in pyridine (1 ml) to which freshly distilled acetic anhydride (0.5 ml) was added. The mixture was kept at room temperature for 2 h. The acetylated compounds were extracted from an aqueous solution with ethyl acetate three times, the extract washed with water, dried over anhydrous Na₂SO₄, and evaporated to dryness under argon. The residue was dissolved in 250 µl of CH₃OH/CH₂Cl₂ (4:1, v/v). Portions (100 µl) were injected for h.p.l.c. analysis.

(v) Methylation of carboxylic acids. The non-saponifiable fraction of the liver extract (250 µl) obtained in (iii) was evaporated to dryness under argon and was redissolved in diethyl ether (1 ml) to which an ethereal solution of diazomethane (1 ml; ca. 15 mg/ml) was added. The solution was kept at room temperature for 30 min. After solvent removal, the residue was dissolved in 250 µl of CH₃OH/CH₂Cl₂ (4:1, v/v), and a portion (100 µl) was injected for h.p.l.c. analysis.

(vi) Action of β-glucuronidase. A portion (200 µl) of selected tissue extracts obtained in (ii) was evaporated to dryness under argon, dissolved in 0.2 M-sodium acetate buffer (2 ml), pH 6.8, and incubated in the presence of bacterial β-glucuronidase (10 mg) for 90 min at 37 °C. The retinoids and other lipids were extracted twice each with hexane and with ethyl acetate. The extracts were pooled, evaporated to dryness under argon, and the residue was dissolved in 200 µl of CH₃OH/CH₂Cl₂. A portion (100 µl) was injected for h.p.l.c. analysis.

(vii) Radioactivity determination. Portions (50–100 µl) of extracts and chromatographic fractions (1 ml) were mixed with 5 ml of Biofluor, and their ³H content was

quantitatively measured by use of an LS-7500 scintillation counter (Beckman Instrument Co., Fullerton, CA, U.S.A.). Background and quench corrections were made by using the H numbers of known solvent mixtures.

Recovery of radioactivity from serum and various tissues was calculated from the total ³H counts (d.p.m.) present in a particular tissue extract relative to the total ³H counts (d.p.m.) in the administered dose.

RESULTS

Distribution of [11-³H]ROG in organs of the rat and in the excreta after its intraperitoneal injection.

After injection (1 h), 13.3 % of the administered dose of radioactivity was present in the serum (Table 1). Thereafter, the percentage in the serum declined to reach a plateau of approx. 2 % from 6 h through to 24 h (Table 1). The amount of radioactivity in the liver was 4.3 % at 1 h and remained at approx. 3 % throughout the 24 h period. The amount of radioactivity in the small intestine rose to a peak of 3.5 % at 6 h and then declined (Table 1), whereas the kidney showed a similar pattern with a peak of 1.2 % at 3 h (Table 1). Small amounts of radiolabelled retinoids were also found in the muscle (0.005 %/g), lung (0.09 %) and adipose tissue (0.023 %/g). During a 24 h period, 3.8 % of the administered dose of radioactivity was excreted in the urine and 2 % in the faeces.

Nature of the radioactive compounds present in serum and tissues

At 1 h, the major labelled retinoids present in the serum were the injected ROG and the newly formed vitamin A esters. Labelled retinol could not be detected (Figs. 2 and 3a). The radioactivity in the ROG peak of serum fell markedly with time and at 24 h was only 8 % of that initially found. The fact that the concentration of ROG in the blood was slightly higher at 24 h than at 3, 6 and 12 h (Fig. 2) is attributed to the variability among the single rats used at each time point. At 24 h, labelled retinol and traces of retinyl esters were also present in the serum (Figs. 2 and 3b).

In a separate set of experiments, the serum retinol values of vitamin A-depleted rats (*n* = 5) increased from

Fig. 2. Time course appearance of radiolabelled metabolites of ROG in various tissues of rats given $[11\text{-}^3\text{H}]\text{ROG}$

Rats were given $50\text{ }\mu\text{g}$ (31 retinol equivalents, or $0.11\text{ }\mu\text{M}$) of $[11\text{-}^3\text{H}]\text{ROG}$ in saline solution by intraperitoneal injection and killed 1, 3, 6, 12 and 24 h after the dose. Serum, liver, small intestine and kidney were analysed by reversed-phase gradient h.p.l.c. for all metabolites. The concentration of each metabolite was determined from the radioactivity associated with each. In the rats studied (290–320 g) measured tissue weights, as a percentage of body weights, were: serum, 4%; liver, 4%; small intestine, 3%; kidney, 0.9%. Abbreviations: POL, polar metabolite; EROL, epoxyretinol; ROL, retinol; EST, vitamin A esters.

Fig. 3. H.p.l.c. profiles of $[11\text{-}^3\text{H}]\text{retinoids}$ from the sera of rats

Samples were analysed 1 h (a) and 24 h (b) after an intraperitoneal dose of $[11\text{-}^3\text{H}]\text{ROG}$. ROL, retinol.

$170 \pm 130\text{ nm}$ to $680 \pm 230\text{ nm}$ after the intraperitoneal administration of 714 nmol of unlabelled ROG. Thus, ROG was hydrolysed in vitamin A-depleted as well as in normal rats.

The level of labelled ROG in the liver was relatively constant ($0.02\text{--}0.05\text{ nmol/g}$) from 1 to 24 h. Except for an unidentified polar fraction present at 1 h, the major radioactive peak at all times was the vitamin A ester fraction (Fig. 2). Thus the decrease in serum vitamin A ester level with time was accompanied by an increase in the vitamin A ester fraction in the liver. Small amounts of retinol and variable amounts of some unidentified labelled compounds (t_R values of 2.0 min and 13.5 min) were also present (Fig. 4a).

ROG was also a prominent peak ($0.02\text{--}0.06\text{ nmol/g}$) in the small intestine (Fig. 2) at all times of study. Significant amounts of retinol (t_R 17.5 min) and of retinyl esters (t_R 25–30 min) as well as several unidentified peaks were also present at most times (Figs. 2 and 5). When an intestinal extract was incubated with bacterial β -glucuronidase, the ROG peak disappeared, and a retinol peak appeared (Fig. 5b). In addition to an unidentified polar fraction, the kidney mainly contained vitamin A esters with some ROG from 1–6 h (Fig. 2). At 12 and 24 h, however, retinol became the major labelled species. Retinol and vitamin A esters predominated in the lung, whereas mainly polar compounds, together with some ROG, retinol and its esters, were found in the muscle. The urine and faeces contained mainly, if not solely, unidentified polar metabolites of ROG.

Fig. 4. H.p.l.c. profiles of $[11\text{-}^3\text{H}]$ retinoids from the liver of a rat

Samples were analysed 24 h after an intraperitoneal dose of $[11\text{-}^3\text{H}]$ ROG. (a) Liver extract; (b) liver extract after hydrolysis with methanolic KOH; (c) liver extract after hydrolysis and treatment with diazomethane; (d) liver extract after hydrolysis and acetylation with acetic anhydride. The presence of retinol (ROL) and its esters is denoted by hatching. RAC, retinyl acetate; ERAC, epoxyretinyl acetate; EROL, epoxyretinol.

Fig. 5. H.p.l.c. profiles of $[11\text{-}^3\text{H}]$ retinoids from the small intestine of a rat

Samples were analysed 3 h after an intraperitoneal dose of $[11\text{-}^3\text{H}]$ ROG. (a) Intestinal extract, (b) intestinal extract after treatment with β -glucuronidase for 1.5 h at 37°C . ROG is hatched in (a); retinol (ROL) is hatched in (b).

Identification of 5,6-epoxyretinol and its ester as metabolites of ROG

In the analysis by h.p.l.c. of liver extracts (Fig. 4a), two discrete unidentified labelled peaks were noted with t_R values of 2.0 min and 13.5 min. The former peak is highly polar (polar compound in Fig. 2), appears in the void volume, and is probably an oxidation product of a labelled metabolite. The latter peak, which appears between ROG and retinol, was greatly enhanced, as was

Fig. 6. H.p.l.c. profiles of $[11\text{-}^3\text{H}]$ retinoids from the liver of a rat dosed with $[11\text{-}^3\text{H}]$ retinyl acetate

Liver extracts were analysed 12 h after a dose of $[11\text{-}^3\text{H}]$ retinyl acetate. (a) Liver extract, (b) liver extract after hydrolysis with methanolic KOH. The presence of retinol (ROL) and its esters is denoted by hatching; EROL, epoxyretinol.

retinol, by hydrolysis of the retinyl ester fraction with base (Fig. 4b). This compound, like retinol, could be acetylated, i.e., it contained an alcoholic group (Fig. 4d), but did not react with diazomethane, i.e., it did not contain a carboxylic acid group (Fig. 4c). Thus, the unidentified non-polar metabolite was not an ester of retinoic acid. Although the presence of a very small amount of retinoic acid cannot be ruled out, the chromatographic behaviours of the parent compound and its acetylated derivative were identical with those of authentic 5,6-epoxyretinol (t_R 13.5 min) and its acetate (t_R 17.5 min) (Figs. 4c and 4d). Other possible metabolic products showed different t_R values, e.g., 4-oxoretinol (6.1 min), 4-hydroxyretinol (11.5 min), and retinoic acid (12.5 min). The amounts of 5,6-epoxyretinol and of retinol formed by hydrolysis of the vitamin A ester fraction of rat liver were roughly equivalent (Fig. 4b).

Upon hydrolysis of the retinyl ester fraction of the intestine, results similar to those found in liver were obtained. In contrast, when $[^3\text{H}]$ retinyl acetate (6.1×10^6 d.p.m. in $10 \mu\text{g}$) was administered to rats under similar conditions, 5,6-epoxyretinyl esters accounted for < 20% of the total retinyl esters present in the liver (Fig. 6).

DISCUSSION

In the stimulation of growth and of cellular differentiation by ROG, a major question is whether it acts

directly or only after its hydrolysis to retinol. Although its direct action on cells cannot be ruled out, ROG clearly can be hydrolysed, presumably by β -glucuronidases in tissues, to retinol when injected *in vivo*. The rate of its hydrolysis is only moderate, however, in that intact ROG was the major component of the serum at 1 h and was clearly evident in serum and tissues at 24 h.

As with retinol, ROG clearly was converted to vitamin A esters that were stored in the liver. The level of ROG did not change appreciably with time in the liver however, whereas it increased and peaked at 6–12 h in the intestine. In the intestine, vitamin A esters were prominent metabolites during the entire period of the study. In the kidney, the levels of ROG and vitamin A esters reached a maximum at 1–3 h, and then decreased with time.

A possible explanation for these time course data is that injected ROG is converted to vitamin A esters in the small intestine and, to some extent, in the kidney. The newly formed vitamin A esters might then be transported in the blood to the liver, where they are stored. Because only one animal was used for each time point, however, this hypothesis clearly requires further validation.

Unlike retinol, the conversion of ROG to vitamin A esters was a relatively slow process. Furthermore, unlike retinol, approx. 50% of the labelled vitamin A esters present in the liver and intestine after the administration of labelled ROG contained 5,6-epoxyretinol. Although retinoic acid is known to form the 5,6-epoxide *in vivo* and *in vitro* (McCormick *et al.*, 1980; McCormick & Napoli, 1982), the formation of 5,6-epoxyretinol and its subsequent esterification have not previously been demonstrated. Why more epoxyretinol is formed from ROG than from retinyl acetate is not clear; possibly, the water-soluble ROG is more accessible to the microsomal epoxidase than is retinol or its esters.

Although 5,6-retinoid epoxides were initially reported to stimulate growth well (Lakshmanan *et al.*, 1965; John *et al.*, 1967; Mallia *et al.*, 1970), later studies indicated that 5,6-epoxyretinoic acid was a weak growth promoter at best (Morgan & Thompson, 1966; Zile *et al.*, 1980). Furthermore, it does not stimulate well the differentiation of vaginal epithelia *in vivo* (Sietsema & DeLuca, 1982). Nonetheless, 5,6-epoxyretinoic acid shows significant activity in some tissue culture systems (Adamo *et al.*, 1979; Strickland, 1979; Newton *et al.*, 1980) and can inhibit chemically induced papilloma and ornithine decarboxylase in mouse skin (Verma *et al.*, 1980). Thus, its formation in significant amounts from ROG is not only of metabolic interest, but also might possibly have physiological importance as well.

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REFERENCES

- Adamo, S., DeLuca, L. M., Akalovosky, I. & Bhat, P. V. (1979) *J. Natl. Cancer Inst.* **62**, 1473–1477
- Barua, A. B. & Olson, J. A. (1985) *J. Lipid Res.* **26**, 1277–1282
- Barua, A. B. & Olson, J. A. (1986) *Am. J. Clin. Nutr.* **43**, 481–485
- Barua, A. B. & Olson, J. A. (1987) *Biochem. J.* **244**, 231–234
- Barua, A. B., Batres, R. O. & Olson, J. A. (1986) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **45**, 832
- Bollenback, G. N., Long, J. W., Benjamin, D. G. & Lindquist, J. A. (1955) *J. Am. Chem. Soc.* **77**, 3310–3315
- Cullum, M. E. & Zile, M. H. (1985) *J. Biol. Chem.* **260**, 10590–10596
- Dunagin, P. E., Zachman, R. D. & Olson, J. A. (1966) *Biochim. Biophys. Acta* **124**, 71–85
- Furr, H. C., Amedee-Manesme, O. & Olson, J. A. (1984) *J. Chromatogr.* **309**, 299–307
- Gallup, J. M., Barua, A. B., Furr, H. C. & Olson, J. A. (1987) *Proc. Soc. Exp. Biol. Med.* **186**, 269–274
- John, K. V., Lakshmanan, M. R. & Cama, H. R. (1967) *Biochem. J.* **103**, 539–543
- Lakshmanan, M. R., Jungalwala, F. B. & Cama, H. R. (1965) *Biochem. J.* **95**, 27–34
- Lippel, K. & Olson, J. A. (1968) *J. Lipid Res.* **9**, 168–175
- Mallia, A. K., John, J., John, K. V., Lakshmanan, M. R., Jungalwala, F. B. & Cama, H. R. (1970) *Indian J. Biochem.* **7**, 102–103
- McCormick, A. M. & Napoli, J. L. (1982) *J. Biol. Chem.* **257**, 1730–1735
- McCormick, A. M., Napoli, J. L., Yoshizawa, S. & DeLuca, H. F. (1980) *Biochem. J.* **186**, 475–481
- Mehta, R. G., Barua, A. B., Olson, J. A. & Moon, R. C. (1987) *Proc. Am. Assoc. Cancer Res.* **28**, 50
- Miller, D. A. & DeLuca, H. F. (1986) *Arch. Biochem. Biophys.* **244**, 179–186
- Morgan, B. & Thompson, J. N. (1966) *Biochem. J.* **101**, 835–842
- Nath, K. & Olson, J. A. (1967) *J. Nutr.* **93**, 461–469
- Newton, D. L., Henderson, W. R. & Sporn, M. B. (1980) *Cancer Res.* **40**, 3413–3425
- Sietsema, W. K. & DeLuca, H. F. (1982) *J. Nutr.* **112**, 1481–1489
- Sklan, D. & Halevy, O. (1984) *Br. J. Nutr.* **52**, 107–114
- Strickland, S. (1979) in *Hormones and Cell Culture* (Sato, G. H. & Ross, R., eds.), vol. 6, part B, pp. 671–676, Cold Spring Laboratory, Cold Spring Harbor, NY
- Verma, A. K., Slaga, T. J., Wertz, P. W., Mueller, G. C. & Boutwell, R. K. (1980) *Cancer Res.* **40**, 2367–2371
- Zachman, R. D., Dunagin, P. E. & Olson, J. A. (1966) *J. Lipid Res.* **7**, 3–9
- Zile, M. H., Inhorn, R. C. & DeLuca, H. F. (1980) *J. Nutr.* **110**, 2225–2230
- Zile, M. H., Inhorn, R. C. & DeLuca, H. F. (1982) *J. Biol. Chem.* **257**, 3537–3543
- Zile, M. H., Cullum, M. E., Simpson, R. U., Barua, A. B. & Swartz, D. A. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2208–2212