Metabolism *in vivo* of all-*trans*-[11-³H]retinoic acid after an oral dose in rats

Characterization of retinoyl β -glucuronide in the blood and other tissues

Arun B. BARUA, Desirée B. GUNNING and James Allen OLSON

Department of Biochemistry and Biophysics, Iowa State University, Ames, IA 50011, U.S.A.

Soon after [11-³H]retinoic acid (RA) (1.1×10^8 d.p.m.) was administered orally to rats either as a large dose (115 μ g = 0.38 μ mol/rat) or mixed with unlabelled RA as a huge dose (22 mg = 73.33 μ mol/rat), retinoyl β -glucuronide (RAG) was identified and characterized as a significant metabolite in the serum and small intestine. Of the administered dose, 70 % remained unchanged as retinoic acid in the stomach up to 1 h. Significant amounts of 5,6-epoxyretinoic acid, 4-hydroxyretinoic acid, esters of retinoic acid and several polar retinoids, including 4-oxoretinoic acid, were also detected in the stomach. No significant difference was observed in the nature of the retinoids found after a large or a huge dose; however, the ratio of RAG/RA was higher after a huge dose than after a large dose. Thus RAG, which is biologically active *in vivo* and *in vitro*, is formed quickly in significant amounts in tissues after a dose of RA.

INTRODUCTION

Retinoic acid (RA) is an important physiological metabolite of retinol in the control of epithelial-cell growth and cellular differentiation (Roberts & Sporn, 1984). Both vitamin A deficiency and large doses of RA markedly affect embryonic development (Kochhar, 1967; Maden & Summerbell, 1986; Gunning *et al.*, 1989). Both physiological and toxic effects may be mediated at the gene level, at least in part, by nuclear retinoic acid receptors (Petkovich *et al.*, 1987; Giguere *et al.*, 1987).

Metabolites of all-*trans*-RA *in vivo* include retinoyl β -glucuronide (RAG), 5,6-epoxyretinoic acid (5,6-ERA), 4-hydroxyretinoic acid (4-HRA) and 4-oxoretinoic acid (4-ORA) (Dunagin *et al.*, 1966; McCormick *et al.*, 1980; Napoli & McCormick, 1981; Zile *et al.*, 1980, 1982; Swanson *et al.*, 1981; Silva & DeLuca, 1982). 4-Oxo-13-*cis*-RA, its glucuronide, and other oxidized metabolites appear in the blood or bile after an oral dose of 13-*cis*-RA (Vane & Bugge, 1981; Vane *et al.*, 1990). In the studies cited above, RA usually was given by intravenous or intraperitoneal injection. However, in many of these past investigations, RAG would not have been extracted by the procedures used.

We recently synthesized RAG from RA chemically (Barua & Olson, 1985a,b, 1989a,b) and, thereafter, demonstrated the endogenous occurrence of RAG in human blood (Barua & Olson, 1986).

The purpose of the present study, therefore, was to examine the metabolites of orally administered doses (both large and huge) of all-*trans*-RA in rats, with particular attention to the formation of RAG and its circulation in the blood. A preliminary communication concerning this study has previously appeared (Barua *et al.*, 1990).

MATERIALS AND METHODS

Chemicals and solvents

All-*trans*-[11-³H]RA was obtained from SRI International, Menlo Park, CA, U.S.A., through the courtesy of the National

Cancer Institute, Bethesda, MD, U.S.A. Other chemicals and solvents used and their purveyors were: all-*trans*-RA and β -glucuronidase from *Escherichia coli* (activity 570 000 units/g) (Sigma Chemical Co., St. Louis, MO, U.S.A.); NaHCO₃, acetone, methanol, dichloromethane, diethyl ether, ethyl acetate and hexane (Fisher Scientific Co., Fair Lawn, NJ, U.S.A.); silica gel for dry column chromatography (Woelm Pharma, Eschewege, Germany, and supplied through Universal Scientific, Atlanta, GA, U.S.A.); glucuronic acid and *p*-chloroperoxybenzoic acid (Aldrich Chemical Co., Milwaukee, WI, U.S.A.).

[11-³H]RA (2 μ Ci) was mixed with unlabelled RA (3 mg) and purified first by column chromatography on a small column of silica gel that was wet-packed with dichloromethane. All-*trans*-RA, which separated from traces of more- and less-polar compounds as a single major yellow band, was eluted with dichloromethane/diethyl ether (1:1, v/v). The all-*trans*-[11-³H]-RA thus obtained, when analysed by isocratic reversed-phase h.p.l.c. as described below, was found to be > 99 % pure. The specific radioactivity was found to be 9.3×10^5 d.p.m./ μ g.

5,6-ERA was prepared by treating methyl retinoate (2 mg) dissolved in diethyl ether (1 ml) with a solution of *p*-chloroperoxybenzoic acid (1 mg) in diethyl ether (1 ml). The crude methyl 5,6-epoxyretinoate was hydrolysed with methanolic NaOH [10 % (w/v); 100 μ l]. After acidification with acetic acid, the retinoids were extracted, then purified by isocratic reversed-phase h.p.l.c. 5,6-ERA [retention time (t_R) 4 min] separated as the major band from another earlier unidentified band (t_R 2 min) and showed a λ_{max} of 321 nm that shifted to 337 nm when a drop of acetic acid was added to the h.p.l.c. solvent. The purified acidic form of 5,6-ERA showed a λ_{max} of 337 nm, which shifted to 308 nm, characteristic of the 5,8-furanoid structure, upon addition of a drop of dilute HCl. ORA and 4-hydroxyretinoic HRA were prepared as described previously (Barua & Ghosh, 1972).

H.p.l.c.

Reversed-phase gradient h.p.l.c. was performed on a Waters Resolve 5 μ m (3.9 mm $\times$ 15 cm) column as described previously (Barua & Olson, 1989a). Reversed-phase isocratic h.p.l.c. was

Abbreviations used: RA, [11-³H]retinoic acid; RAG, retinoyl β -glucuronide; 5,6-ERA, 5,6-epoxyretinoic acid; 4-HRA, 4-hydroxyretinoic acid; 4-ORA, 4-oxoretinoic acid; t_R , retention time.

carried out in a similar way, but a solvent mixture of methanol/water (7:3, v/v), containing 10 mM-ammonium acetate, at a flow rate of 1.2 ml/min, was used.

Retinoic acid solution for oral dosing

A methanolic solution containing [$^{11}\text{-}^3\text{H}$]RA (9.3×10^5 d.p.m./ μg) in a small test tube was dried under a stream of argon, and the residue was dissolved in ethanol and diluted with an equal volume of peanut oil. The mixture was vortex-mixed to obtain a uniform clear solution, which contained $0.38 \mu\text{mol}$ (1.1×10^8 d.p.m./ $100 \mu\text{l}$). This solution ($100 \mu\text{l}$) was fed to rats as a large dose. The huge dose was made by vortex-mixing $73.3 \mu\text{mol}$ of unlabelled RA in $400 \mu\text{l}$ of ethanol/peanut oil (1:5, v/v) to which $100 \mu\text{l}$ of the small dose was added. The huge dose was a thick yellow pasty solution. In both instances, the same amount of radioactivity (1.1×10^8 d.p.m.) was given to each rat.

Animal studies

Sprague-Dawley rats weighing about 300 g were fed on a normal rat diet. Large doses of the retinoid were given to male rats and huge doses on day 10 of gestation to female pregnant rats, which were concomitantly used for study of teratogenicity. The rats were dosed by means of a Microman positive-displacement pipette (Gilson Medical Electronics, Villiers-le-Bel, France; supplied by Rainin Instruments Co., Woburn, MA, U.S.A.). The volumes of large and huge doses (containing the same amount of radioactivity) were $100 \mu\text{l}$ and $500 \mu\text{l}$ respectively, which were pipetted well down into the throats of the animals. Another $50\text{--}100 \mu\text{l}$ of peanut oil were pipetted subsequently into the mouth to rinse the dose down. The rats were anaesthetized with diethyl ether 0.5 or 1 h after the dose, the chest was cut open, and blood was collected from the heart by means of a syringe. Liver, stomach, small intestine, large intestine and kidneys were collected and kept frozen at -72°C until analysis. Blood was allowed to clot for about 15 min and then was centrifuged for 20 min at 1500 rev./min (500 g). Serum was separated and, whenever possible, analysed on the same day; otherwise, it was kept frozen at -72°C .

Extraction of retinoids

Serum (0.5 ml) and about 1 g portions of liver, kidney, small intestine and stomach (together with its contents) were extracted with a mixture of dichloromethane, ethyl acetate and methanol as described previously (Barua & Olson, 1989a). The extracts were dissolved in methanol/dichloromethane [3:1 (v/v); $100\text{--}500 \mu\text{l}$], and portions ($50\text{--}100 \mu\text{l}$) were analysed by h.p.l.c.

Control experiments

To determine whether the retinoids formed were artefacts of the extraction and isolation procedure, control experiments were conducted by using residual tissues after complete extraction of radioactivity. Thus [^3H]RA solution (10^8 d.p.m.) in methanol was added to residual liver and intestinal tissues that had been mixed previously with five times their weight of anhydrous Na_2SO_4 . The mixture was ground, and the retinoids were extracted exactly the same way as described under 'Extraction of retinoids' above. The extract was then analysed by h.p.l.c.

Other procedures

Action of β -glucuronidase. Serum ($200\text{--}500 \mu\text{l}$), or its extract, and other tissue extracts in $50 \mu\text{l}$ of methanol were diluted with phosphate-buffered saline (0.15 M-sodium phosphate/0.15 M-NaCl, pH 7.5; 0.5 ml) and incubated with β -glucuronidase (5 mg) at 37°C for 1 h. Retinoids were extracted and analysed as described under 'Extraction of retinoids' above.

Determination of radioactivity. Portions ($50\text{--}100 \mu\text{l}$) of extracts from serum or other tissues were each mixed with 5 ml of biodegradable-grade Scintiverse BD (Fisher). The h.p.l.c. eluates, collected as 1 ml fractions, were allowed to evaporate in a fume hood for 3–4 h. The residual solution (~ 0.2 ml) was mixed with 5 ml of Scintiverse BD. The ^3H content was then quantitatively measured by use of an LS-7500 scintillation counter (Beckman Instruments Co., Fullerton, CA, U.S.A.). Background and quench corrections were made by using H number values of known solvent mixtures (Beckman Instruments Co., 1979).

Recoveries of radioactivity from serum and other tissues were calculated on the basis of the total ^3H radioactivity (d.p.m.) present in a particular extract relative to the total ^3H radioactivity (d.p.m.) in the administered dose.

RESULTS

When a solution of all-*trans*-[$^{11}\text{-}^3\text{H}$]RA was fed orally to rats, the radioactivity, although remaining largely in the stomach up to 1 h, was found in small amounts in the blood and various tissues within 30 min of dosing. The distribution of radioactivity in serum and in other tissues of rats 30 min and 1 h after the administration of either a large dose ($0.38 \mu\text{mol}/\text{rat}$) or a huge dose ($73.3 \mu\text{mol}/\text{rat}$) of RA is shown in Table 1.

Distribution and nature of radioactive compounds in serum and other tissues

Stomach. As indicated in Table 1, most (88–95%) of the recovered radioactivity was present in the stomach and its contents. Although the dose of [^3H]RA largely remained intact (Table 2), radioactive 5,6-ERA, 4-HRA and 4-ORA were also detected and characterized. Two other non-polar radioactive compounds, one tentatively characterized as an ester of RA, were also present (Table 2). However, no RAG was detected in the stomach.

Serum. Although [^3H]RA was found to circulate in the blood within 30 min of dosing (Table 3), the predominant radioactive peak found at that time was a polar compound that was eluted close to the solvent front during h.p.l.c. Although the identity of this compound was not established with certainty, 4-ORA exhibits similar h.p.l.c. behaviour. At 1 h after a large or huge dose, however, RA was a predominant compound detected in the blood. Small quantities of 4-HRA and 5,6-ERA (Table 3) were also identified in the blood. Retinoyl β -glucuronide (RAG) was found to be the major metabolite ($15.2 \text{ nmol}/\text{ml}$) (Table 3; Fig. 1a) 1 h after a huge dose of RA and a significant metabolite (6 nmol of RAG/ml) in the serum 30 min after a large dose. Non-polar radioactive compounds (t_R 18–28 min; Fig. 1c), possibly esters of RA, were found in the serum 1 h after either dose of RA.

Small intestine. Irrespective of the size of the dose, [^3H]RA was the major compound in the small intestine (Table 3). Among all the tissues, however, the highest concentration of RAG was found in the small intestine (Table 3). Smaller quantities of 5,6-ERA, 4-HRA, 4-ORA mixed with polar compounds, and a non-polar compound, presumably an ester of RA, were also present in the small intestine (Table 3).

Liver. The pattern of radioactive compounds found in the liver after a large or huge dose was similar (Table 3). At both 30 min and 1 h, RA predominated, followed by a polar fraction, which probably included 4-ORA. A significant amount of 5,6-ERA and a small quantity of RAG were also detected.

Kidney. After a $0.38 \mu\text{mol}$ dose, kidney radioactivity was low (0.1%) at 30 min, but was much higher (2.5%) after 1 h (Table 1). At both doses, administered RA was the major radioactive compound present at 30 min or 1 h after the dose (Table 3).

Table 1. Recovery of radioactivity from serum and other tissues 30 min and 1 h after a large or huge dose of [³H]RA

Rats were fed orally 0.38 μmol or 73.3 μmol of [11-³H]RA in ethanol/peanut oil and killed 30 min or 1 h after the dose. The retinoids were extracted, and the radioactivity associated with each organ was determined. The volume of serum was assumed to be 4 % of the body weight.

Tissue	Dose ... Time ...	Recovery (%)		
		Large		Huge
		30 min	1 h	1 h
Stomach + contents,		64.7	69.7	51.9
Small intestine		1.6	5.1	1.5
Liver		1.5	1.2	1.3
Serum		0.2	1.1	0.8
Kidney		0.1	2.5	0.1
Total ...		68.1	79.6	55.6

Table 2. Distribution of ³H-labelled retinoic acid and its metabolites in the stomach and its contents of rats 30 and 60 min after oral doses of 0.38 and 73.3 μmol , each containing 1.1×10^8 d.p.m. radioactivity

Compound	Dose (μmol) ... Time (min) ...	Content (nmol/g of tissue)		
		0.38		73.3
		30	60	60
Retinoic acid		28	26	5700
5,6-ERA		5.4	4.4	830
4-ORA*		2.0	1.9	270
4-HRA		2.1	1.1	280
Non-polar esters†		4.1	4.6	400
RAG		ND‡	ND	ND

* Mixed with polar metabolites.

† Partially characterized.

‡ ND, not detected.

Small quantities of 4-ORA, 5,6-ERA, and 4-HRA were also detected in the kidney. However, RAG was not found in the kidneys within 1 h of dosing (Table 3).

Characterization of metabolites in blood and other tissues

Retinoyl β -glucuronide. The t_R of radiolabelled RAG in blood and other tissues was identical with that of standard RAG in at least two different h.p.l.c. procedures. The t_R values for RAG and RA in the gradient h.p.l.c. system were 9.8 and 11.8 min respectively. In certain serum or tissue extracts where RAG was present as a minor metabolite, especially after a large dose, RAG appeared as a shoulder on the RA peak in the gradient h.p.l.c. procedure. To confirm that the peak was indeed due to RAG, and not due to *cis*-RA, isocratic h.p.l.c. using methanol/water (7:3, v/v) at a flow rate of 1.2 ml/min was used. Under these conditions, RAG (t_R 7 min) separated very well from both *cis*-RA (t_R 13.5 min) and *trans*-RA (t_R 16 min) (Figs. 2a and 2b).

The u.v.-absorption spectrum of RAG, obtained from serum as a metabolite after a huge dose of RA, was identical with that of standard RAG (λ_{max} at 360 nm in h.p.l.c. solvent) (Fig. 2c).

The identity of RAG, obtained after a large or huge dose, was further confirmed by treating the serum, its extract or tissue extracts, with β -glucuronidase. After treatment, both the RAG peak in the h.p.l.c. profile (Fig. 1b) and the radioactivity associated with it (Fig. 1c) disappeared, and the area of the RA peak and the radioactivity associated with it correspondingly increased (Figs. 1b and 1c).

5,6-ERA, 4-HRA and 4-ORA. The identity of these metabolites was established from their retention time in both isocratic and gradient h.p.l.c. procedures and by examination of spectra of fractions collected after a huge dose. The ionic form of 5,6-ERA, both in the standard and from an extract of the stomach and its contents, exhibited λ_{max} at 321 nm in the h.p.l.c. solvent (Fig. 2d). The maximum shifted to 308 nm in the presence of a trace of HCl, owing to rearrangement of the 5,6-epoxide form to the 5,8-furanoid form or to 337 nm in the presence of acetic acid, due to neutralization of the charge (Fig. 2d). Both standard 4-HRA and a retinoid isolated from stomach and its contents showed λ_{max} at 340 nm in the h.p.l.c. solvent mixture (Fig. 2e). Similarly, both standard 4-ORA and a derivative of RA isolated from stomach and its contents exhibited λ_{max} at 355 nm (Fig. 2e).

Table 3. Distribution of [³H]RA and its metabolites in the serum, small intestine, liver and kidney of rats 30 min and 60 min after oral doses of 0.38 and 73.3 μmol

Compound	Tissue ...	Content											
		(pmol/ml)			(pmol/g of tissue)								
		Serum			Small intestine			Liver			Kidney		
		30	60	60	30	60	60	30	60	60	30	60	60
	Dose (μmol) ...	0.38	0.38	73.3	0.38	0.38	73.3	0.38	0.38	73.3	0.38	0.38	77.3
RA		17	56	13800	410	1190	79300	120	163	46700	114	2130	5400
5,6-ERA		7	14	2700	40	206	1300	35	31	9700	20	155	600
4-ORA*		26	35	3500	81	210	11100	88	66	15000	43	447	1050
4-HRA		ND†	12	3700	29	100	5000	16	9	Trace	ND	265	1900
RAG		6	14	15200	82	167	17000	20	20	Trace	ND	ND	ND
Non-polar esters†		ND	6	6050	37	110	5100	ND	ND	1000	21	340	ND

* Mixed with polar metabolites.

† Partially characterized.

‡ ND, not detected.

Fig. 1. Reversed-phase gradient h.p.l.c. profiles of (a) serum (500 μ l) retinoids 1 h after the administration of a huge (73.3 μ mol) oral dose of [3 H]RA to a rat, showing the appearance of RAG in blood, (b) the same serum (150 μ l) after treatment with β -glucuronidase, showing the conversion of RAG into RA, and (c) radioactivity associated with serum (500 μ l and 150 μ l) before (—) and after (---) treatment with β -glucuronidase respectively

Note: absorption units full scale for (a) and (b) are 0.256 and 0.08 respectively. Abbreviation: ROL, retinol.

Fig. 2. Reversed-phase isocratic h.p.l.c. profiles and absorption spectra

(a) and (b) Reversed-phase isocratic h.p.l.c. profiles of (a) standard retinoids and (b) serum retinoids 1 h after a large (0.38 μ mol) dose of [3 H]RA, showing the appearance of [3 H]RAG in the blood of a rat. The mobile phase was methanol/water (7:3, v/v) containing 10 mM-ammonium acetate; the flow rate was 1.2 ml/min. (c-e) Absorption spectra of retinoid fractions (in h.p.l.c. solvent) separated by gradient h.p.l.c. from the blood and stomach of a rat 1 h after a huge (73.3 μ mol) dose of [3 H]RA. (c): —, RAG from blood; —, RA from blood; (d): —, ERA from stomach; ---, same after treatment with acetic acid; ---, same after treatment with HCl; (e): —, ORA from stomach; ---, HRA from stomach. Additional abbreviations: cRA, *cis*-retinoic acid; tRA, all-*trans*-retinoic acid.

Because of the co-elution of ORA with unidentified compounds that absorb strongly at shorter wavelengths, the small peak at 280 nm, characteristic of the 4-oxo group, was masked. Thus the exact concentration of 4-ORA could not be determined. The presumed presence of non-polar esters of RA was based on the t_R of standard methyl retinoate during h.p.l.c. and on the disappearance of radioactivity in tissue extracts from this region after alkaline hydrolysis. Because of the very small amounts involved, further characterization of these presumed esters was not possible.

DISCUSSION

Retinoyl glucuronide was first recognized as a metabolite of retinoic acid in the bile (Olson, 1968; Zile *et al.*, 1980) and small intestine of the rat (Zile *et al.*, 1982). Recently, after a 80 mg dose of 13-*cis*-RA, the presence of small amounts of the glucuronide conjugate of 13-*cis*-RA along with other oxidized and glucuronidated metabolites in human bile has been reported (Vane *et al.*, 1990). With the availability of synthetic RAG (Barua & Olson, 1985a, 1989a), the earlier finding that RAG

supported the growth of vitamin A-deficient rats (Nath & Olson, 1967) was confirmed (Barua & Olson, 1985a). RAG, like RA, induces the differentiation of HL-60 cells, but is less cytotoxic (Gallup *et al.*, 1987; Zile *et al.*, 1987; Janick-Buckner *et al.*, 1991) and less teratogenic (Gunning *et al.*, 1989, 1990) than RA. The occurrence of RAG in human blood as an endogenous compound has been clearly demonstrated (Barua & Olson, 1986), but the appearance of RAG in the blood after administration of RA has not previously been reported.

In the present study we have been able to show that RAG is synthesized rapidly from administered RA and can be detected in the blood within 30 min after the administration of RA. The ratio of RAG/RA in the serum was much higher after a huge dose (1.10) of RA than after a large dose (0.25). After a large dose of RA, the RAG peak was best resolved from RA and other metabolites by isocratic h.p.l.c.

The highest concentration of RAG was found in the small intestine (Table 3). In this regard, Zile *et al.* (1982) earlier found that the small intestine was a major site for the biosynthesis of RAG from RA. Although we demonstrated previously that RAG was the major retinoid in the liver up to 24 h after an intraperitoneal dose of [³H]RAG (Barua & Olson, 1989a), only very small amounts were found after oral dosing with RA (Table 3). In the present study, we could not detect any RAG in the kidney within 1 h after the dose of RA.

After the oral administration of a large dose of RA, radioactivity recovered from the blood and the major tissues (e.g. liver, small intestine and kidney) accounted for only 3.5–9.9% of the total dose (Table 1). To locate the rest of the administered radioactivity, the stomach and its contents were extracted and analysed. Indeed, 64–70% of the administered radioactivity, which equalled 88–95% of the total recovered radioactivity, was still present in the stomach 0.5–1 h after the dose. H.p.l.c. analysis of the extract from the stomach and its contents showed that, besides administered RA, three other radioactive compounds (namely 4-ORA, 4-HRA and 5,6-ERA) were present in amounts sufficient for characterization by their u.v. spectra. Furthermore, 5,6-ERA was characterized by demonstrating the expected shifts in its absorption maximum in the presence of acetic acid and HCl. Thus the 5,6-epoxy ring clearly does not isomerize to the furanoid form in the presence of stomach acid.

5,6-ERA was previously characterized as a physiological metabolite of RA (McCormick *et al.*, 1980, 1982; McCormick & Napoli, 1982; DeLuca *et al.*, 1981; Leo *et al.*, 1984) on the basis of its molecular ion (*M*⁺), which is the same for both the 5,6- and the 5,8-epoxide of RA. In our studies, the small intestine and its contents also contained lesser amounts of 5,6-ERA. Whether 5,6-ERA, 4-ORA and 4-HRA found in the intestine and other tissues originated primarily *in situ* or were derived in part from those formed in the stomach merits further study.

As far as we are aware, this is the first study where the stomach and its contents have been analysed after a dose of RA. It seems unlikely that 5,6-RA, 4-ORA, 4-HRA and some non-polar esters are produced metabolically in stomach cells. Moreover, because a total of < 1% of oxidized derivatives of RA were formed in our inactivated-control experiments, it seems unlikely that their formation can be attributed to an artefact of the process of extraction and analysis. Thus the most plausible explanation is that they are formed in the stomach by a process of chemical

oxidation. Because RA is only a very minor component of foods, whether ingested retinyl ester, the major dietary form of vitamin A, is affected similarly merits attention.

These studies were supported by grants from the Competitive Research Grants Program, Science and Education Administration, United States Department of Agriculture (87-CRCR-1-2320), and the National Institutes of Health (DK 32793, DK 39733 and CA 46406). This is Journal Paper no. J-14178 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, U.S.A.; Project 2534.

REFERENCES

- Barua, A. B. & Ghosh, M. C. (1972) *Tetrahedron Lett.* **18**, 1823–1825
- Barua, A. B. & Olson, J. A. (1984) *J. Lipid Res.* **25**, 304–309
- Barua, A. B. & Olson, J. A. (1985a) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **44**, 772
- Barua, A. B. & Olson, J. A. (1985b) *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. (1989a) *Biochem. J.* **263**, 403–409
- Barua, A. B. & Olson, J. A. (1989b) *U.S. Pat.* 4855463
- Barua, A. B., Gunning, D. B. & Olson, J. A. (1990) *FASEB J.* **4**, A660
- Beckman Instruments Co. (1979) *Beckman Operations Manual*, LS 7500 Liquid Scintillation System, Beckman Instruments Co., Fullerton, CA
- DeLuca, H. F., Zile, M. & Sietsema, W. K. (1981) *Ann. N.Y. Acad. Sci.* **359**, 25–36
- Dunagin, P. R., Zachman, R. D. & Olson, J. A. (1966) *Biochim. Biophys. Acta* **124**, 71–85
- Gallup, J. M., Barua, A. B., Furr, H. C. & Olson, J. A. (1987) *Proc. Soc. Exp. Biol. Med.* **186**, 269–274
- Giguere, V., Ong, E. S., Segui, P. & Evans, R. M. (1987) *Nature (London)* **330**, 624–629
- Gunning, D., Barua, A. B. & Olson, J. A. (1989) *FASEB J.* **3**, A467
- Gunning, D., Barua, A. B. & Olson, J. A. (1990) *FASEB J.* **4**, A660
- Janick-Buckner, D., Barua, A. B. & Olson, J. A. (1991) *FASEB J.* **5**, 320–325
- Kochhar, D. M. (1967) *Acta Pathol. Microbiol. Scand.* **70**, 398–404
- Leo, M. A., Iida, S. & Lieber, C. S. (1984) *Arch. Biochem. Biophys.* **234**, 305–312
- Maden, M. & Summerbell, D. (1986) *J. Embryol. Exp. Morphol.* **97**, 239–250
- McCormick, A. M., Napoli, J. L., Yoshizawa, S. & DeLuca, H. F. (1980) *Biochem. J.* **186**, 475–481
- McCormick, A. M. & Napoli, J. L. (1982) *J. Biol. Chem.* **257**, 1730–1735
- Napoli, J. L. & McCormick, A. M. (1981) *Biochim. Biophys. Acta* **666**, 165–175
- Nath, K. & Olson, J. A. (1967) *J. Nutr.* **93**, 461–469
- Olson, J. A. (1968) *Vitam. Horm. (N.Y.)* **26**, 1–63
- Petkovich, M., Brand, N. J., Krust, A. & Chambon, P. (1987) *Nature (London)* **330**, 444–450
- Roberts, A. B. & Sporn, M. B. (1984) in *The Retinoids*, vol. 2 (Sporn, M. B., Roberts, A. B. & Goodman, D. S., eds.), pp. 209–286, Academic Press, New York
- Silva, D. P. & DeLuca, H. F. (1982) *Biochem. J.* **206**, 33–41
- Swanson, B. N., Frolik, C. A., Zaharevitz, D. W., Roller, P. P. & Sporn, M. B. (1981) *Chem. Pharmacol.* **30**, 107–113
- Vane, F. M. & Bugge, C. J. L. (1981) *Drug Metab. Dispos.* **9**, 515–520
- Vane, F. M., Bugge, C. J. L., Rodriguez, L. C., Rosenberger, M. & Doran, T. I. (1990) *Xenobiotica* **20**, 193–207
- Zile, M. H., Schnoes, H. K. & DeLuca, H. F. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3230–3233
- Zile, M. H., Inhorn, R. C. & DeLuca, H. F. (1982) *J. Biol. Chem.* **257**, 3544–3550
- 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