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The role of vitamin A status in the conversion of all-*trans* retinoyl β-glucuronide to retinoic acid in male Sprague-Dawley rats

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Orally administered all-trans retinoyl β-glucuronide, after its absorption from the intestine, is distributed widely in the tissues, except for brain, of both vitamin A-sufficient (A+) and vitamin A-deficient (A−) rats. Although the digestion and rate of absorption of retinoyl β-glucuronide are similar in A+ and A− rats, the rates of hydrolysis of retinoyl β-glucuronide to retinoic acid markedly differ. Thus, after an oral dose (6.3 μmol) of all-trans retinoyl β-glucuronide in corn oil, retinoic acid peaks at 4 hr in the plasma at a concentration of 2.5 to 4.2 μM in A− rats, up to 40-fold higher than in A+ rats. The peak retinoic acid concentration in A− rats increased with the severity of the deficiency. Similarly, the ratio of retinoic acid/retinoyl β-glucuronide in various tissues of A− rats is 1.3 to 12.5-fold higher than in those of A+ rats. In the absence in tissues of retinol or its ester, A− rats clearly use administered retinoyl β-glucuronide as a ready source of retinoic acid. (J. Nutr. Biochem. 9:8–16, 1998)

Keywords: retinoyl β-glucuronide; retinoic acid; retinol; metabolism; vitamin A status

Introduction

All-*trans* retinoyl β-glucuronide (RAG) is a prominent water-soluble metabolite of all-*trans* retinoic acid (RA) in humans and in other animal species.[1–3] RAG and RA show many similarities in properties as well as some key differences. Both promote the growth of vitamin A-deficient rats,[4] induce the differentiation of several cell lines,[5–8] are efficacious in treating acne,[9] and are absorbed and excreted in a similar fashion when applied topically to rat skin.[10] In contrast to RA, however, RAG is not cytotoxic to cells in culture.[5–8] Orally administered RAG, even at very large doses, is not teratogenic in pregnant rats.[11] Subcutaneously injected RAG, however, is rapidly hydrolyzed in pregnant mice to RA, which is teratogenic.[12] RAG does not bind either to retinoid-binding proteins[13] or to nuclear retinoic acid receptors.[14]

It is speculated that β-glucuronides of endogenous, biologically active compounds play several physiologic roles. Their formation may inactive the compound and increase its rate of urinary excretion (detoxification), may enhance its biologic activity and toxicity[15,16] may serve a transport function[2,3,16] or may be involved in ligand transfer.[3,15]

In vitamin A-sufficient rats, intraperitoneally or orally administered RAG remains intact in sera and other tissues with very little hydrolysis to RA.[11,18] On the contrary, when RAG is administered orally to vitamin A-deficient rats, RAG is hydrolyzed to RA, which appears in the serum as its major metabolite.[19] Thus, the uptake and metabolism of RAG seems to depend on the method of administration and on the vitamin A status of the animal. We consequently have investigated in greater detail the effect of the vitamin A status of rats on the uptake of orally administered RAG by tissues and on its hydrolysis to RA.

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Methods and materials

Chemicals and solvents

Methanol, dichloromethane, dichloroethane, acetonitrile, and ethyl acetate were supplied by Fisher Scientific Co. (Fair Lawn, NJ, USA). For high-performance liquid chromatography (HPLC), HPLC-grade solvents were used.

Retinoids

All-*trans* retinoic acid was a gift from BASF (Parsippany, NJ USA). All-*trans* retinoyl β-glucuronide was synthesized by a new procedure[19] from all-*trans* retinoic acid. In brief, RA was treated with 1,1′-carbonyldiimidazole in pyridine to give retinoyl imidazole. The tetrabutylammonium salt of glucuronic acid was then added to the reaction mixture, followed by NAH, to produce RAG in 60 to 80% yield. The product was purified by high-performance liquid chromatography (HPLC). The RAG preparation, which contained some α-anomer, was used as such. Retinol (ROL) and retinyl palmitate were purchased from Sigma Chemical Co. (St. Louis, MO USA). Retinoids were analyzed for purity by using absorption spectroscopy and reverse-phase HPLC, as described below. When necessary, the retinoids were again purified before use by HPLC.

The dose of RAG was prepared as follows: The required amount of RAG was weighed and ground to a fine powder in a porcelain mortar. A measured quantity of corn oil was added, and the mixture was ground well to obtain a solution. The oily solution was pipetted into a vial. The mortar was washed twice with measured amounts of fresh oil, which were pipetted into the same vial. The final concentration of RAG was 6.3 μmol/120 μL oil.

Rats

Although the metabolism of retinoids is not known to differ in male and female rats, only male Sprague-Dawley rats ($n = 20$) weaned at day 21 were used in this study. The rats were divided into three groups and housed in individual cages. One group ($n = 9$), the vitamin A-sufficient group (A+), received normal rat chow and the second group ($n = 9$), denoted A−, received a vitamin A-deficient diet (Diet No. 904646, ICN, Cleveland, OH USA). The third group ($n = 2$), raised on normal rat chow and denoted N+, was used to determine endogenous retinoid levels. Although a synthetic diet supplemented with vitamin A would have been a better control diet than rat chow, which is undefined in composition, the probability that our findings are not directly related to vitamin A status is remote. All of the rats were given water and diet ad libitum. The weights of the rats were recorded twice weekly.

At the end of week 4 on the diet, three rats each from the A+ and A− groups were fed 120 μL of the oil containing 6.3 μmol RAG by means of a Gilson positive displacement pipette (Rainin Instruments, Woburn, MA USA). Blood was collected from the tail vein of each rat before dosing and 1, 2, 3, and 4 hr after the dose. The rats were sacrificed at 5 hr, and blood and other tissues were collected as described below. This same procedure was used to administer RAG and to collect blood and other tissues from the remaining rats raised on A+ ($n = 3$) and A− ($n = 3$) diets at the end of week 5 and at the end of week 6. The two N+ rats, raised on the A+ diet but not dosed with RAG, were sacrificed at the same time (6 weeks) as the last group of dosed A+ rats. Blood and other tissues were collected, as indicated below.

Collection of blood and other tissues

After making small cuts in the tail vein with a sharp razor blade, the four hourly postdosing blood samples (250 μL each) were collected in 5 or 6 microhematocrit capillary tubes (75 mm long), obtained from Fisher Scientific Co. Blood was centrifuged in a hematocrit centrifuge, and the plasma was removed by means of a syringe. The pooled plasma was analyzed immediately, or kept frozen at −20°C until analysis within a week.

The rats were killed under ether anesthesia 5 hr after the dose. An incision was made from the stomach to the breast. Blood was collected from the aorta by means of a syringe. The blood was centrifuged immediately to obtain plasma. The liver, kidneys, spleen, heart, lung, brain, and a sample of skeletal muscle were removed and washed with saline to free the tissues from any adhering blood. All contents of the stomach and intestines were removed. The small intestines were flushed with saline. The tissues and gastrointestinal contents were kept frozen at −20°C. Samples were analyzed as soon as possible, but no later than 2 months after freezing.

Extraction and characterization of retinoids

Retinoids in plasma were extracted by a slight modification of the procedure described earlier.[18] In brief, 50 μL of plasma were vortexed first with ethanol (100 μL), which contained retinyl acetate as the internal standard, and then with ethyl acetate (200 μL) in the presence of 10 μL of acetic acid (10%, v/v). After centrifugation, the supernatant solution was pipetted out and kept cold. The pellet was vortexed with hexane (100 μL), and then centrifuged. The supernatant solutions were pooled, vortexed with water (200 μL), and then centrifuged. The upper organic phase was removed carefully, and the solvent was evaporated under a gentle stream of argon. The residue was dissolved in a mixture of methanol/dichloromethane (3:1, v/v, 50 μL). An aliquot of 40 μL was analyzed by HPLC, as described below.

Retinoids in tissues were extracted by a modification of published procedures[20,21] as follows: portions of liver (0.1 to 0.3 g); intestines (3 to 5 g); skeletal muscle (0.5 to 1 g); and the whole organs of kidneys, spleen, heart, brain, and lung were finely minced. Each tissue, or portion thereof, was homogenized with 3 to 5 mL of a mixture of isopropanol/dichloromethane (ISP/DCM) (2:1, v/v). In some representative samples, ISP/DCM containing retinyl acetate as internal standard was used to determine the rate of recovery. The homogenates were transferred quantitatively to a glass vial (final volume = 10 mL) and then left at −20°C under argon overnight. On the next day, the vials were vortexed briefly and returned to the freezer. On the third day, the extract from each tissue, except liver, was filtered into a 25 to 50 mL conical distilling flask. The residual tissues were washed once with ISP/DCM, and filtered; the pooled filtrate was then evaporated in a rotary evaporator at 37°C under reduced pressure. In the case of the liver, only a 1-to-2 mL aliquot of the extract was processed. The solvent from the aliquot was evaporated to dryness under argon. The residue from each tissue was dissolved in ISP/DCM (2:1, v/v) (200 μL), transferred into a conical microcentrifuge tube (500 μL), and centrifuged for 15 sec in a microcentrifuge. An aliquot (20 to 30 μL) of the supernatant solution was analyzed by HPLC.

All of the stomach and intestinal contents were transferred to a glass flask (50 mL), mixed thoroughly with ISP/DCM (15 to 40 mL, depending on the amount of the contents), and kept at −20°C overnight. On the next day, the mixture was stirred briefly and returned to the freezer. On the third day, an aliquot (1 to 2 mL) of the solution was evaporated under argon, and the residue was dissolved in ISP/DCM (100 μL). An aliquot (20 to 30 μL) was then analyzed by HPLC.

All of the reported retinoids (ROL, RAG, RA, and retinyl esters) in plasma, stomach and intestinal contents, and other tissues of rats were identified not only by their behavior during HPLC, but also from their characteristic UV spectra as measured by a

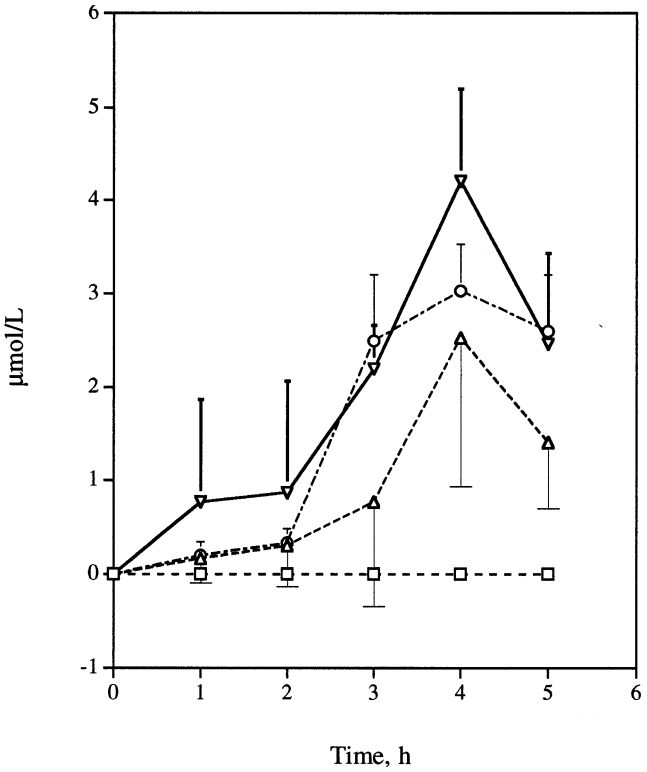

Figure 1 Plasma concentrations of retinoic acid at various times after an oral dose of retinoyl β-glucuronide. Results are expressed as means ± SD. A+ rats (n = 9) □; A− rats, 4 weeks (n = 3) Δ; A− rats, 5 weeks (n = 3) ○; A− rats, 6 weeks (n = 3) ∇.

photodiode array detector (PDA). Distinction between the *trans* and *cis* isomers of the metabolites was not made. The concentrations of the retinoids were measured from peak areas as well as from the observed optical densities in their UV spectra, as recorded by the photodiode array detector.[21]

HPLC

For the analysis of plasma retinoids, a reverse-phase gradient procedure[18] was done using Waters (Milford, MA USA) components; namely, a refrigerated autosampler (WISP Model 717), an automated gradient controller, two pumps (Model 510), a photodiode array detector (PDA) (Model 996), a Millenium 2010 Chromatography Manager, a NEC Powermate (Model 486/33i), and a Hewlett-Packard Laser Jet III printer. Two solvents were employed: methanol:water (68:32, v/v, containing 10 mM ammonium acetate) (solvent A) and methanol:dichloromethane (4:1, v/v) (solvent B). A 20-min linear gradient of solvent A (100%) to solvent B (100%) was followed by isocratic elution with solvent B for an additional 10 min. At the end of the run, the solvent system was restored to its initial condition by using a 5-min linear gradient from solvent B to solvent A. The column was allowed to equilibrate for 10 min with solvent A before the next run. A Waters 5 μm Resolve 15 cm column was used at a flow rate of 1.2 ml/min.

Retinoids in tissues were analyzed in a similar manner with a Model 991 PDA detector and compatible accessories. In this instance, a Rainin Microsorb-MV 3 μm 10-cm column (Rainin Instrument Co., Woburn, MA USA) was used with a 15-min linear gradient of methanol:water (3:1, v/v) containing 10 mM ammonium acetate to methanol:dichloromethane (4:1, v/v) at a flow rate of 0.8 ml/min. This modification resulted in better separation of tissue retinoids.

Results

Plasma retinoid concentrations before dosing with RAG

As expected, rats raised on normal rat chow (A+ rats) weighed more (average wt, 245 ± 10, 305 ± 1, and 303 ± 7 g at weeks 4, 5, and 6, respectively) than rats raised on a vitamin A-deficient diet (A− rats) (average wt, 179 ± 4, 226 ± 16, and 237 ± 7 g at weeks 4, 5, and 6, respectively). The A− rats clearly were in a "plateau" phase of growth between 5 and 6 weeks, well before marked physiologic changes and rapid weight loss ensued. The mean plasma retinol concentrations of A+ rats (n = 3/wk) were 2.34 ± 0.47, 3.32 ± 0.15, and 3.24 ± 0.13 μM after wk 4, 5, and 6, respectively, whereas the concentrations of plasma ROL of A− rats (n = 3/wk) were 0.23 ± 0.16, 0.10 ± 0.15, and 0.10 ± 0.15 μM, after weeks 4, 5, and 6, respectively. Thus, vitamin A reserves were clearly depleted in the A− group of animals. Endogenous plasma concentrations at 6 weeks of RA and RAG in the two N+ rats not dosed with RAG were below the detection limits (≤0.1 μM RA and ≤0.08 μM RAG), primarily because only small volumes of plasma were analyzed.

Plasma RA and RAG concentrations in response to an oral dose of RAG

After an oral dose of 6.3 μmol RAG to A− rats, RA appeared in the plasma within 1 hr. Its concentration rose to a peak at 4 h (2.5 ± 1.6, 3.0 ± 0.50 and 4.2 ± 1.0 μM at weeks 4, 5, and 6, respectively) (*Figure 1*) and then declined. In contrast, RA concentrations in the plasma of A+ rats remained at or below the detection limits (≤0.1 μM) (*Figure 1*). In A− rats, plasma RAG concentrations remained very low or undetectable, primarily because only small volumes of plasma were analyzed. Although RAG could occasionally be detected in the plasma of A+ rats, the pattern of its appearance with time was not orderly. HPLC chromatograms of plasma retinoids in representative A+ and A− rats before and after dosing with RAG are shown in *Figures 2* and *3*. In all A+ rats, endogenous ROL (peak 3) was the major peak (*Figure 2*). RAG (peak 1), when present, was in much higher concentrations in A+ rats than RA (peak 2), which was always very minor (*Figure 2B*). In contrast, RA (peak 2) was the major peak in A− rats, whereas both RAG (peak 1) and retinol (peak 3) peaks were minor (*Figure 3B*). The spectra recorded by the PDA detector for retinoids in peaks 1, 2, and 3 were identical with the spectra recorded for authentic RAG, RA, and ROL, respectively.

Retinoids in other tissues

Retinol and retinyl esters in tissues of dosed (A+ and A−) and undosed (N+) rats. Mean concentrations of retinol and retinyl esters in the three groups of rats are given in *Table 1*. In A+ (n = 9) and N+ (n = 2) rats, liver clearly is the major storage organ, with over 90% of the total recovered vitamin A. Kidney, small intestine, lung, and heart generally contained small but significant amounts (0.5 to 3.0%), whereas spleen, brain, and muscle were much

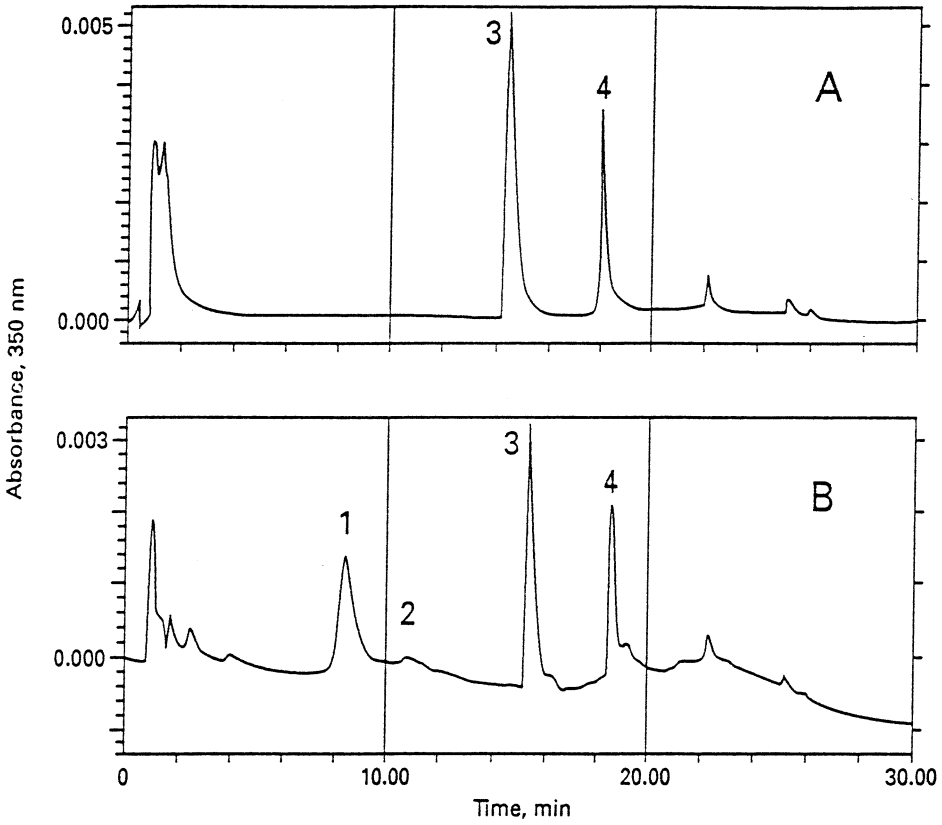

Figure 2 Gradient HPLC chromatogram of plasma retinoids in vitamin A-sufficient rats (A+) before (A) and 4 hr (B) after an oral dose of retinoyl β-glucuronide. Labeled peaks are: 1: RAG; 2: RA; 3: retinol; 4: retinyl acetate as the internal standard.

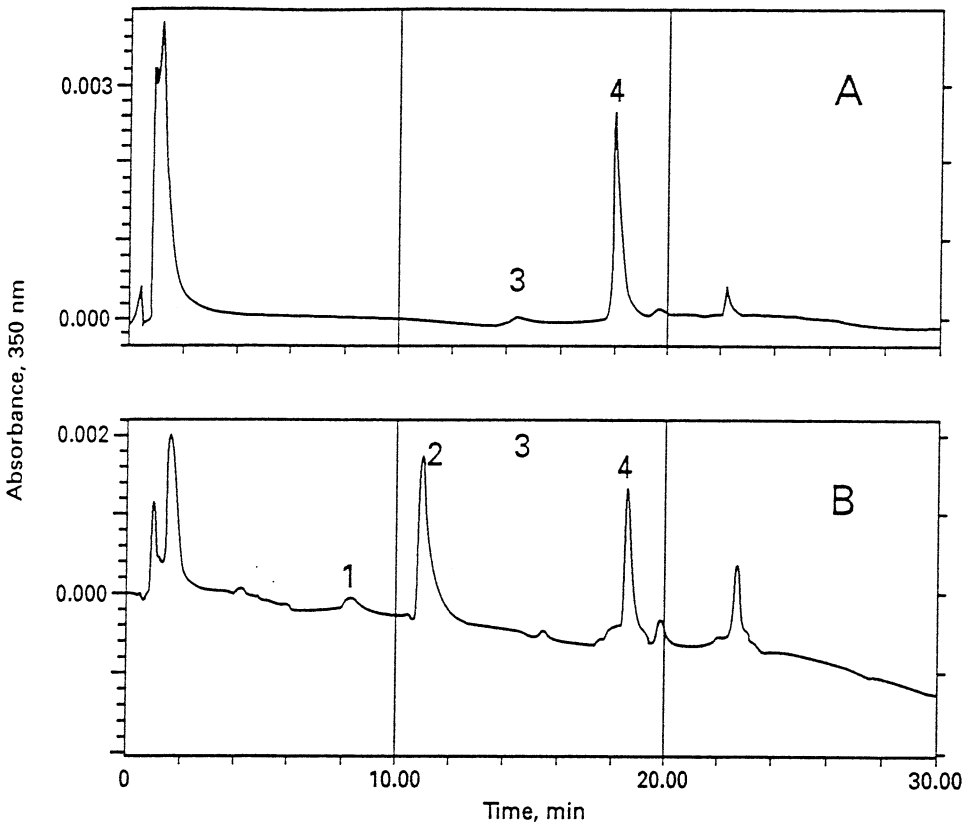

Figure 3 Gradient HPLC chromatogram of plasma retinoids in vitamin A-deficient rats (A−) before (A) and 4 hr (B) after an oral dose of retinoyl β-glucuronide. Labeled peaks are: 1: RAG; 2: RA; 3: retinol; 4: retinyl acetate as the internal standard.

Table 1 Retinol (ROL) and retinyl esters (ROLE) in tissues (nmol/g) of dosed (A+ and A−) and undosed (N+) rats*

Retinoid	Group	*n*	Liver	Kidney	Small intestine	Spleen	Lung	Heart	Brain	Muscle
ROL	A+	9	416 (124)	5.4 (1.7)	nd	nd	16.9 (2.7)	1.2 (0.5)	0.8 (0.2)	0.8 (0.5)
	N+	1	33.1	trace	nd	3.2	2.5	10.7	1.0	0.5
		1	22.5			2.2	1.7	3.7	0.4	0.2
	A−	9	70.5 (31.9)	0.1 (0.1)	nd	nd	nd	nd	nd	nd
ROLE	A+	9	862 (148)	nd	nd	nd	nd	nd	nd	nd
	N+	1	1388	26.2	38.9	nd	43.8	8.4	0.3	trace
		1	1114	18.8	27.9			23.0	2.6	0.1
	A−	9	trace	nd	nd	nd	nd	nd	nd	nd

*For A+ and A− rats, mean values are given ± SD (in parentheses). Values of tissues from rats sacrificed at 4, 5, and 6 weeks are pooled in their analysis. For undosed rats (N+), values for both animals are given. Not detected, nd.

lower (<0.3%). Under the conditions of analysis, RAG, RA, and retinoyl esters were not detected in tissues of undosed (N+) rats, nor were any retinoids found in their stomach and intestinal contents. Whereas the mean total liver vitamin A in N+ and A+ rats was similar (1380 and 1278 nmol/g, respectively), the percentage of ROL was much higher in A+ rats (33%) than in N+ rats (2%). Whereas some ROL and traces of retinyl esters were detected in the livers of A− rats, these were not detected in other tissues of A− rats.

RAG and its products in the gastrointestinal contents. Five hours after dosing orally with 6.3 μmol RAG, a large percentage (57% and 66%) of the administered dose of RAG and its products was found in the gastrointestinal contents of both A+ and A− rats, respectively (*Table 2*). With a dose of this magnitude, the rate of intestinal absorption was clearly limited.

Nonetheless, no significant differences existed in the amount absorbed between A+ and A− rats ($P > 0.25$). In both A+ and A− rats, however, RAG was similarly hydrolyzed to RA (42% and 39%, respectively) and transesterified to as yet unidentified retinoyl esters (17% and 24%, respectively) (Peak 5, *Figure 4*). It is not clear whether these two reactions were enzymatic in nature or were acid-catalyzed.

Table 2 RAG and its products (total nmol) in the gastrointestinal contents of A+ ($n = 9$) and A− rats ($n = 9$)*

Retinoid	A+	A−
Retinoyl β-glucuronide	1500 ± 640	1530 ± 240
Retinoic acid	1510 ± 880	1620 ± 330
Retinoyl ester	600 ± 860	990 ± 530
Retinol	nd	nd
Retinyl ester	nd	nd
Total recovery	3610 ± 2380	4140 ± 1100
Percent of dose	57% ± 38%	66% ± 17%

*Results are expressed as means ± SD.

RAG and RA in tissues of dosed (A+ and A−) rats. In both A+ and A− rats, RAG and RA were found in all tissues studied except in brain, where only very small amounts of RA were detected (*Table 3*). The total amounts of RAG + RA in tissues of A+ and A− rats were comparable, although most tissues of A− rats contained more than those of A+ rats.

The ratio of RA to RAG was universally higher on a tissue-by-tissue basis, however, in A− rats (0.22 to 2.63) than in A+ rats (0.12 to 0.99) (*Table 3*). The distribution of retinoids in the livers of A+ and A− rats is shown in *Figures 5* and *6*, respectively. Retinoyl esters (nmol/g) were found in only three tissues, the intestine (36 ± 12 for A−, 3.3 ± 1.5 for A+), the lung (5.5 ± 3.2 for A−, 3.9 ± 1.3 for A+) and the heart (0.3 ± 0.2 for A−, 1.0 ± 0.8 for A+).

Characterization of compounds

RAG isolated from plasma and tissues was characterized by being treated with β-glucuronidase, which yielded RA. The latter's identity was confirmed by its HPLC behavior and by its spectrum. RA isolated from plasma and tissues was treated with diazomethane, which yields methyl retinoate. The latter's identity was also confirmed by its HPLC behavior and by its spectrum. No attempts were made to distinguish between *trans* and *cis* isomers of the metabolites.

The gradient HPLC/spectrum index plot of an extract from the gastrointestinal contents is shown in *Figure 4*. A major peak (peak 5) appeared where standard ethyl or methyl retinoate generally eluted. The UV spectrum of the compound (spectrum 5, *Figure 4*) was identical with that of retinoyl esters. Saponification of this retinoid resulted in RA, whereas incubation of the retinoid with β-glucuronidase did not produce RA or any other compounds. This peak constituted a major retinoid in the gastrointestinal contents and was found as well in extracts of the intestine, lung, and heart. Its HPLC behavior, UV spectra, and properties are consistent with those of a retinoyl ester.

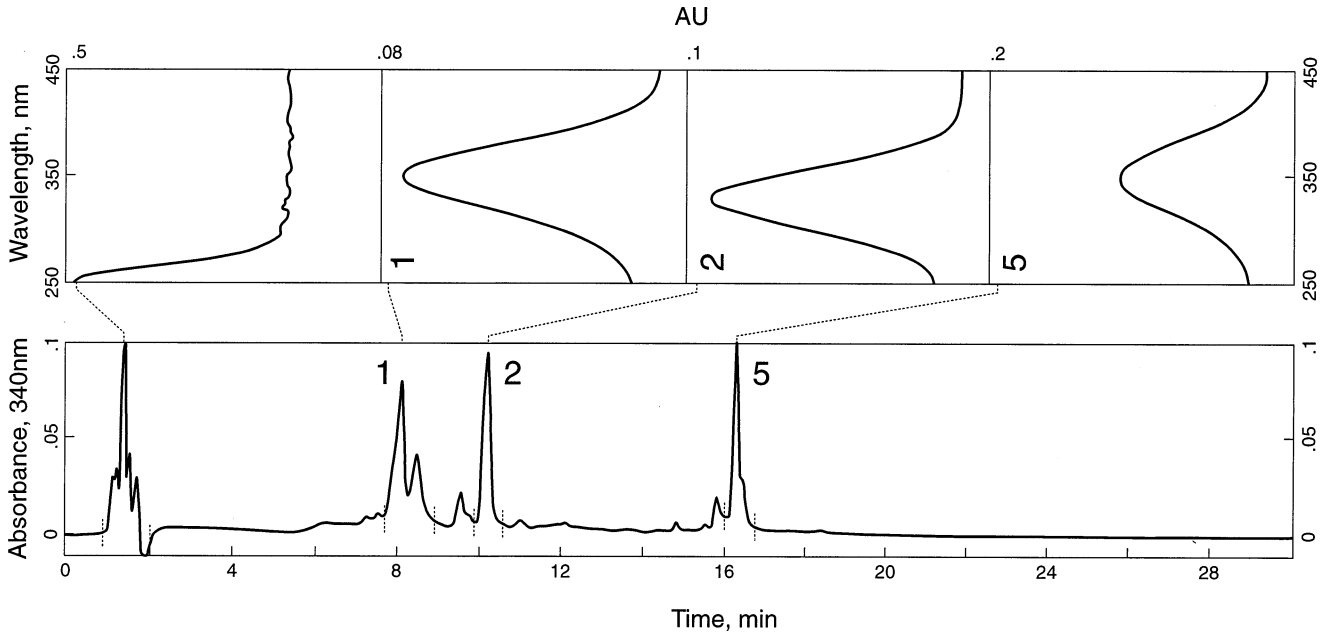

Figure 4 Gradient HPLC spectrum index plot of an extract of stomach and intestinal contents of a vitamin A-deficient rat (A−) 5 hr after an oral dose of retinoyl β-glucuronide. The labeled peaks are: 1: RAG; 2: RA; 5: unidentified retinoyl esters.

Discussion

When a large dose (6.3 μmol) of RAG is administered orally to rats, approximately 1/3 of the dose is absorbed from the GI tract within 5 hr. Within the GI tract, however, two major reactions occur: 1) hydrolysis of approximately 40% of RAG to RA and 2) transesterification of approximately 20% of RAG to retinoyl esters. Whether the hydrolysis is enzymatic or acid-catalyzed is not clear. Because acyl-glucuronides can readily transfer the acyl group to other molecules,[15,22,23] transesterification reactions might be expected. In all likelihood, however, this reaction in the gastrointestinal contents is acid-catalyzed. Presumably, all three of these compounds are absorbed, although possibly at different rates, from the intestinal tract. An important observation, however, in the context of this study, is that the relative amounts of these three compounds as well as their total amounts in the GI contents were the same in both A+ and A− rats. Thus, vitamin A status does not appreciably influence either of these gastrointestinal processes.

RAG was widely distributed among tissues at 5 hr after dosing, being found in all tissues examined, except in brain. Understandably, the small intestine and liver contained the highest RAG concentrations. After dosing with RAG, RA was even more widely distributed, being found, albeit in low concentrations, in brain as well as in the other tissues. Tissues of both A+ and A− rats contained both RAG and RA.

The primary difference between A+ and A− rats was in the relative amounts of RA and RAG present. Four hours after dosing with RAG, for example, the RA concentrations in the blood plasma was 2.5 to 4.2 μM in A− rats but ≤0.1 μM in A+ rats, a 25- to 42-fold increase (*Figure 1*). In this regard, the peak RA concentration increased with the degree of depletion from 4 to 6 weeks. Similarly, the RA/RAG ratio in A− rats was always higher for a given tissue than the ratio in A+ rats (*Table 3*).

The most likely explanation of these observations is that tissues that are depleted of vitamin A convert all available

Table 3 The mean concentrations of RAG and RA, in nmol/g, in the tissues of A+ (n = 9) and A− (n = 9) rats 5 hr after dosing with RAG*

Tissue	A+ rats			A− rats			Ratio of RA/RAG in A−/A+
	RA	RAG	RA/RAG	RA	RAG	RA/RAG	
Liver	14.4 ± 19.3	14.6 ± 18.2	0.99	60.3 ± 15.7	22.9 ± 21.6	2.63	2.66
Kidney	nd	nd	—	1.1 ± 0.3	0.45 ± 0.4	2.44	—
Small intestine	3.9 ± 1.8	12.4 ± 3.3	0.31	55.9 ± 25.9	135 ± 82	0.41	1.32
Spleen	0.7 ± 1.0	2.9 ± 1.8	0.23	2.2 ± 1.8	3.6 ± 2.7	0.61	2.65
Lung	0.4 ± 0.4	3.4 ± 2.2	0.12	1.4 ± 1.0	6.5 ± 4.8	0.22	1.83
Heart	1.1 ± 0.8	13.3 ± 4.5	0.08	2.1 ± 0.7	2.1 ± 2.1	1.00	12.5
Brain	nd	nd	—	1.0 ± 0.1	nd	—	—
Muscle	trace	trace	—	1.8 ± 1.7	trace	—	—

*Results are expressed as means ± SD.

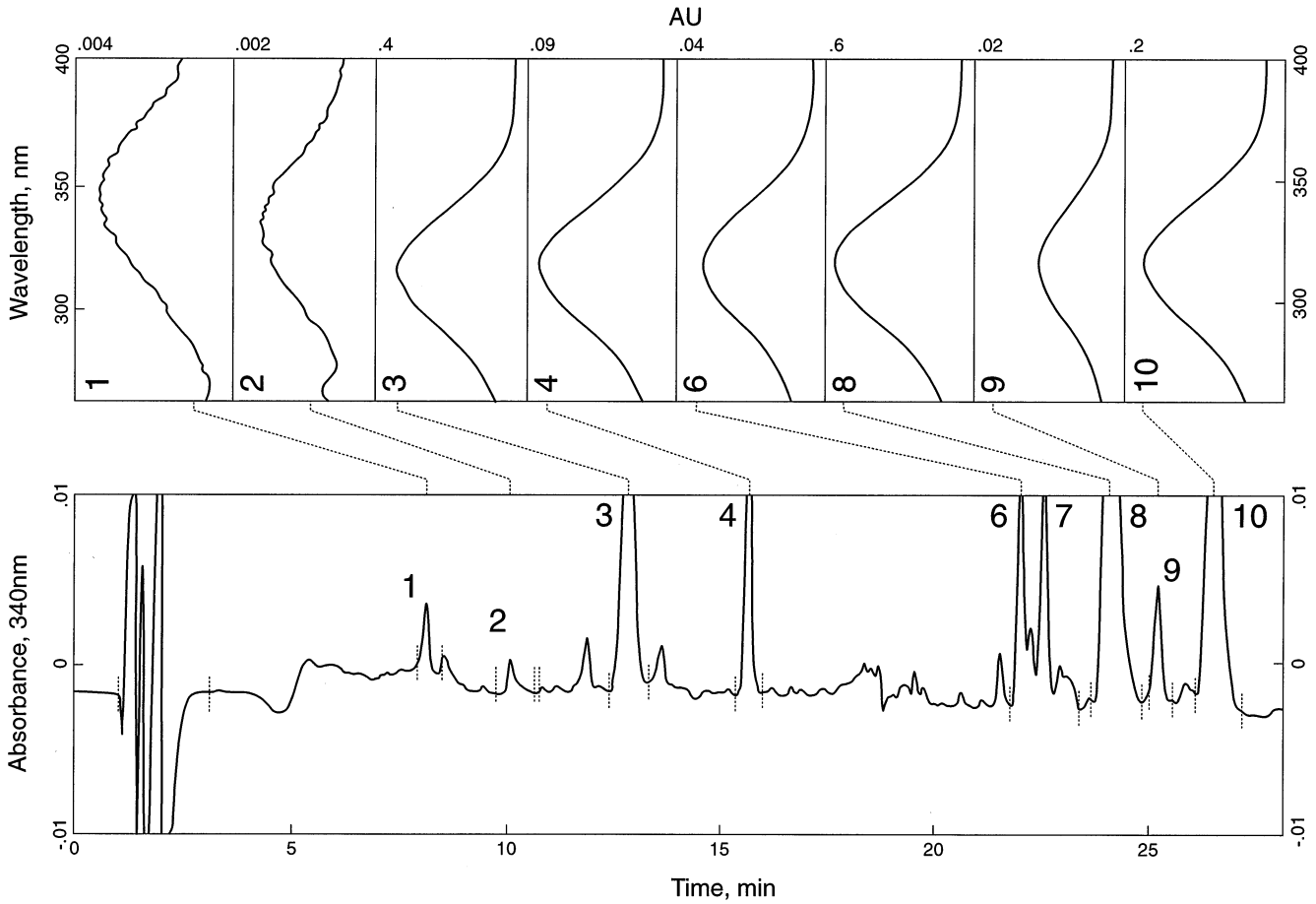

Figure 5 Gradient HPLC spectrum index plot of liver retinoids in a vitamin A-sufficient rat (A+) 5 hr after an oral dose of retinoyl β-glucuronide. Labeled peaks are: 1: RAG; 2: RA; 3: ROL; 4: retinyl acetate as the internal standard; 6–10: retinyl esters.

sources of retinoids to RA, its most biologically active metabolite. Because retinol is essentially absent from all tissues except liver in A− animals (*Table 1*), administered RAG serves as a ready source of RA. RAG is normally present in human plasma and, after a dose of RA, is a major metabolite in the intestinal mucosa.[1,3] Indeed, in recent in vitro studies with cell organelles from tissues of A+ and A− animals, the hydrolysis of RAG to RA is significantly more rapid in the A− preparations.[24] The reservoir of RAG in the body is probably similar to that of RA, inasmuch as plasma concentrations are similar (4 to 16 nmol/L)[1] and tissue concentrations of RA, which are higher than in plasma,[25,26] seem to be roughly matched by those of RAG.[3] Thus, the reservoir of RAG, although not large, is clearly significant relative to the needs for RA in a depleted animal.

The nature of the β-glucuronidases that act on RAG in various tissues is now being explored. The mode of administration also plays a role in the metabolism of RAG. When given orally, very large doses of RAG are not teratogenic in rats,[11] but when injected subcutaneously in mice, they are.[12] The key difference is that orally administered RAG is not rapidly hydrolyzed to RA, whereas subcutaneously and intravenously injected RAG is. RA is well known to be highly teratogenic.[11,12] Thus, the mode of administration markedly influences the rate of RAG hydrolysis.

After an oral dose of RAG, nonpolar esters of RA were found in appreciable amounts in the gastrointestinal contents and to a limited extent in the intestine, lung, and heart, but not in other organs. Acyl-glucuronides are known to transfer their acyl groups readily to acceptor molecules, both enzymatically[22] and nonenzymatically.[23] Although retinoylated proteins have been detected in the nuclei of cells both in vitro[27] and in vivo,[28] RAG has not been identified thus far as the acyl donor.

Thus, when necessary, RAG can serve as a source of RA. Furthermore, the rate of its conversion to RA depends on the vitamin A status of the animal. The finding that, after an oral dose, RAG can be detected in most tissues of both vitamin A-sufficient as well as vitamin A-deficient rats points toward the possible involvement of RAG in the transport and storage of retinoic acid in target tissues.

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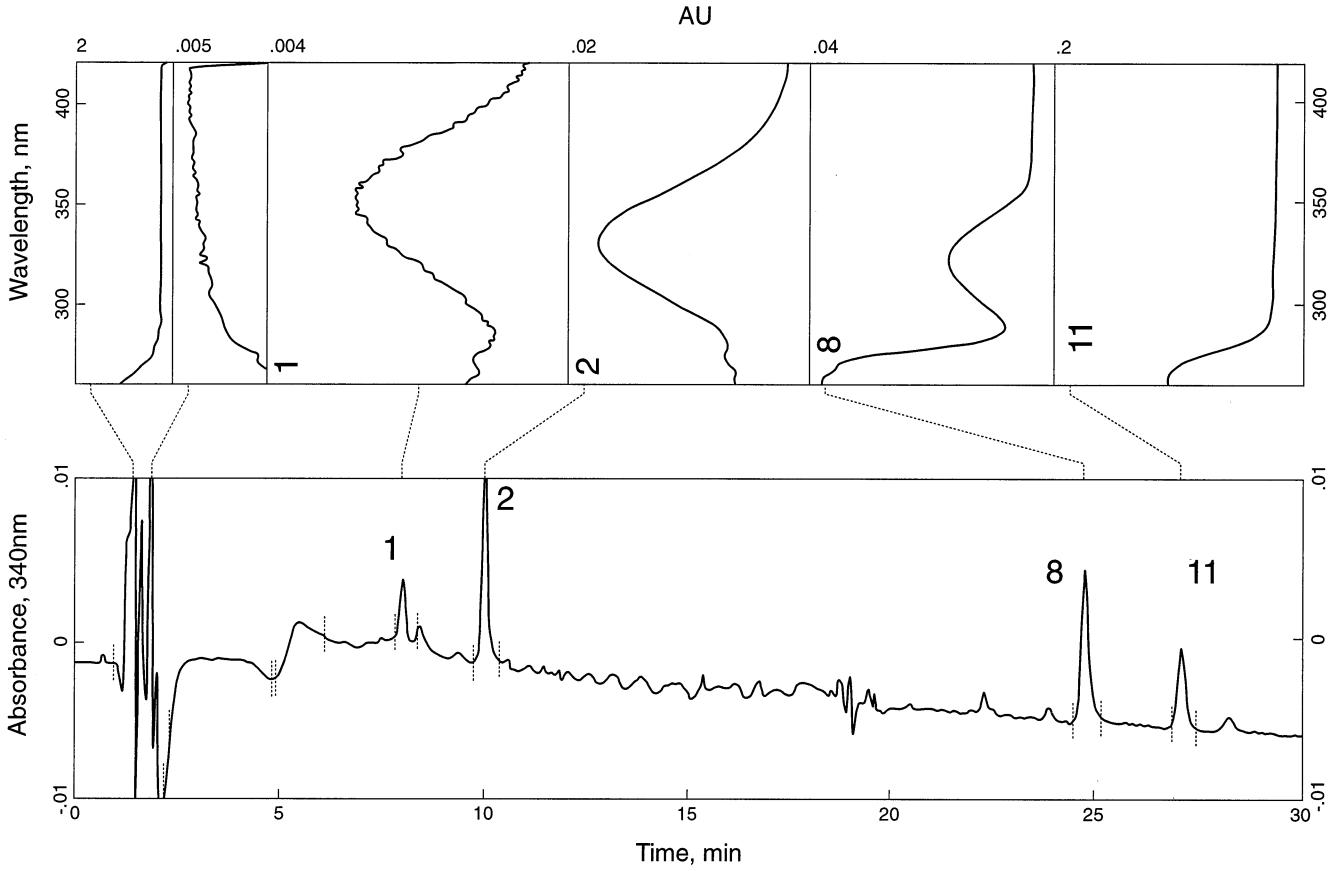

Figure 6 Gradient HPLC spectrum index plot of liver retinoids in a vitamin A-deficient rat (A−) 5 hr after an oral dose of retinoyl β-glucuronide. Labeled peaks are: 1: RAG; 2: RA; 8: retinyl ester; 11, unidentified nonvitamin A peak.

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