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DOSE-DEPENDENT KINETICS OF ALL-*TRANS*-RETINOIC ACID IN RATS

PLASMA LEVELS AND EXCRETION INTO BILE, URINE, AND FAECES

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Abstract—Plasma concentration–time curves for all-*trans*-retinoic acid (RA) after 0.015, 0.25 or 5 mg/kg, i.v., deviated from first-order kinetics in the rat. Within 10 min after the i.v. infusion, a rapid, dose-dependent decrease in RA concentration was observed (slope steepest at the lowest dose). During a secondary phase of slower decline, the times required to halve the RA concentration after 0.015, 0.25 and 5 mg/kg were 40, 65 and 120 min respectively. At later times, the concentration–time curves for all three dose levels assumed a fast rate of decline (half-life about 19 min at the lower dose). The dose-dependent kinetics of RA in plasma were not due to enterohepatic recirculation of RA, since RA levels in plasma were not lower in rats with biliary fistulas given comparable doses. In contrast, circulating levels of RA metabolites remained elevated for several hours and were significantly diminished by interruption of the enterohepatic circulation. After a dose of [10-3H]RA, the rate of biliary excretion of radiolabeled material was initially slower after 5 mg/kg RA than after 0.015 mg/kg RA. Within the first 24 hr, however, approximately the same proportion of both doses appeared in bile. All-*trans*-retinoyl-β-glucuronide is only a minor biliary metabolite of RA. Glucuronidation of RA was dose-dependent, since the percentage of total biliary metabolites represented by all-*trans*-retinoyl-β-glucuronide increased with increasing dose. Renal excretion of RA and its metabolites was significantly decreased by interruption of the enterohepatic circulation. The percentage of dose excreted in the urine decreased with increasing dose.

All-*trans*-retinoic acid (RA) is a biologically potent metabolite of retinol (vitamin A_1) [1]. It sustains body growth and differentiation of epithelial tissues, but, unlike retinol, does not support visual and reproductive functions [2–4]. Metabolites of RA have been shown to cause reversal *in vitro* of squamous metaplasia in tracheas from vitamin A-deficient hamsters [5]. Thus, the physiology and pharmacodynamics of RA may be a function of the relative abundance of RA and its metabolites. A clear understanding of the kinetics of RA biotransformation, including the quantitative importance of various metabolic pathways, cannot be derived from currently available data. Previous studies on RA disposition have employed a wide range of doses, often without consideration of possible dose-dependent alterations in the pharmacokinetics and metabolite pattern of RA [6–11]. It is now well recognized that, for some chemicals, rates of metabolism and excretion into bile and urine can be significantly altered by changes in dose level [12–15]. Furthermore, in several of the studies on RA, animals that were symptomatically ill as a result of vitamin A deficiency were used [11, 16–18]. Data from these experiments were most likely influenced by loss of normal, retinoid-induced enzymes [19] and by decreased food consumption [20].

This present work explores the effects of dose on the kinetics of RA and its metabolites in the plasma, bile, urine, and feces of normal rats.

MATERIALS AND METHODS

Sources of RA. Nonradioactive RA was supplied by Hoffman–La Roche, Inc., Nutley, NJ. This material was found to be 99 per cent pure by high-pressure liquid chromatography (h.p.l.c) with u.v. detection at 280 and 365 nm. All-*trans*-[10-^{3}H]Retinoic acid (sp. act. 1.11 Ci/mmole) was synthesized by the New England Nuclear Corp., Boston, MA, under National Cancer Institute Contract NO 1 CP 75937. This material was 95 per cent pure as shown by high pressure liquid chromatographic radioassay. Crystalline RA was stored over liquid nitrogen, and solutions of RA were kept at −20° in an argon atmosphere. To avoid isomerization of RA, all laboratory procedures were executed under yellow light.

Animals. Male Sprague–Dawley rats were obtained from Taconic Farms (Germantown, NY) and maintained on Wayne Lab-Blox (Allied Mills, Chicago, IL) and water *ad lib.* for 2–7 days prior to use. Each animal weighed 285 and 315 g on the day of experimentation. Each rat was anesthetized with diethyl ether, and a catheter (Silastic, medical grade, 0.02 in i.d. × 0.037 in o.d., Dow Corning Corp., Midland, MI) was introduced into the right atrium via the right external jugular vein. The bile ducts of half of the animals were catheterized with polyethyl-

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ene tubing (No. 10). The catheters were externalized through a midscapular incision and inserted into the lumen of a slender, metal spring that was firmly attached to the back of the animal and, at the distal end, to a swiveling device on a cage top. Each animal received 50 μl of a freshly prepared dimethyl sulfoxide solution of [10-^{3}H]RA (0.015, 0.25 or 5 mg/kg; diluted with unlabeled RA to approximately 17 μCi/rat) in a 30-sec infusion into the left external jugular vein. Each rat was placed in an individual metabolism cage designed for the separation of urine and feces and had free access to laboratory feed and water throughout the experiment. Bile samples were subjected immediately to analysis by h.p.l.c. or stored at −85° in an argon atmosphere. Venous blood samples (0.40 ml) were periodically withdrawn and centrifuged immediately for 2 min in heparinized, 1.5-ml centrifuge tubes in a Brinkmann model 3200 table-top centrifuge. Plasma samples (0.20 ml) were lyophilized to dryness in a Reactivial (5 ml, screwcap, Pierce chemical Co., Rockford, IL) and then assayed for content of RA and RA metabolites. The cellular component of blood was resuspended in normal saline and returned to the animal as soon as possible after each sampling procedure. Patency of the catheter was maintained by flushing it with a small volume of heparinized saline. Animals were killed 24 hr after RA administration, and cages were rinsed with 40 ml water. Feces, urine, and cage rinses were stored at −85° until analysis for content of radioactivity.

Assays for RA and RA metabolites in plasma. A specific assay for RA was devised using h.p.l.c. High concentrations of RA could be measured with an in-line u.v. detector, whereas low concentrations required the use of tritiated RA of high specific activity. Each lyophilized plasma sample was extracted with 0.30 ml methanol (spectrograde, Burdick & Jackson, Muskegon, MI), and then centrifuged at 9000 *g* for 8 min at 4°. Seventy-five microliters of the extract was analyzed in a Spectra-Physics model 8000 HPLC equipped with a Partisil 10/25 ODS reverse-phase column (Whatman, Inc., Clifton, NJ), a Spectra-Physics model 8210 UV detector with a 365 nm wavelength filter, and an electronic peak integrator for the determination of peak areas. The column was maintained at 28°; the mobile phase consisted of a 55/45 mixture (v/v) of acetonitrile/1% ammonium acetate (flow, 2 ml/min). Eluate fractions were collected at 1-min intervals and then evaporated to dryness in a Vortex-Evaporator (Buchler, Fort Lee, NJ). Fractions were redissolved in 0.2 ml of 90% NCS tissue solubilizer (Amersham/Searle, Arlington Heights, IL) and mixed with 10 ml Econofluor (New England Nuclear Corp.). Radioactivity was measured in a Packard model 3255 scintillation counter; absolute disintegrations per minute were calculated using external standardization. The quantity of RA was calculated from the amount of radioactivity associated with the RA peak and the initial specific activity of the dose. Percent recovery for the extraction procedure was determined by comparing u.v. detector response to on-column RA standards with the response to RA standards that had been extracted from fresh rat plasma. Recovery was also examined by adding [10-^{3}H]RA to fresh plasma and measuring the amount of label that was extracted into methanol.

To quantify total radioactivity (i.e. total RA plus its metabolites) per plasma sample, 0.20 ml of the initial 0.30 ml methanol extract was removed from each extraction vial, and each plasma sample was reextracted with 3 ml of fresh methanol. Fifty microliters of the first extract and the entire second extract were evaporated to dryness in separate scintillation vials. Radioactivity in residues was determined as described above. Total radioactivity per plasma sample was calculated as the label in 0.20 ml of the first extract plus the label in the second extract.

*Isolation and identification of all-*trans*-retinoyl-β-glucuronide (RAG).* Bile from rats that had received intravenous RA (5 mg/kg) was subjected to h.p.l.c. using a reverse-phase column (Spherisorb 5 μm ODS, Spectra-Physics) and gradients of acetonitrile and 1% ammonium acetate. For rapid isolation of large quantities of one particular metabolite, an isocratic system consisting of 50/50 acetonitrile/1%ammonium acetate (flow, 2 ml/min) was used. High pressure liquid chromatographic eluates containing the metabolite were lyophilized to dryness, and the metabolite was redissolved in methanol. A u.v. spectrum of the metabolite was obtained in a Cary model 118 spectrophotometer (scan, 450 to 230 nm). The metabolite could not be satisfactorily chromatographed on normal phase columns due to extensive peak broadening. Further, initial efforts to obtain a mass spectrum of the metabolite using the direct probe were unsuccessful. To resolve these problems, the metabolite was subjected to two different methylation procedures. One method employed dimethylsulfinyl carbanion and methyl iodide while the other employed diazomethane. Derivations were executed according to methods published previously [21, 22]. Products of the reactions were first separated by reverse-phase h.p.l.c. (Spherisorb 5 μm ODS column; acetonitrile/1% ammonium acetate gradient, 55/45 to 80/20 in 15 min; flow, 2 ml/min), and then further purified by normal-phase h.p.l.c. (Whatman Partisil PXS 5/25 column; ethyl acetate/hexane mobile phase). Purified substances were analyzed, using direct inlet probes, in a JEOL model JMS-01SG-2 mass spectrometer in the electron impact mode at 70 eV ionization voltage. The metabolite was incubated with β-glucuronidase (Type B-3 from bovine liver, Sigma Chemical Co., St. Louis, MO) according to the method of Fishman *et al.* [23].

Quantification of RA and RA metabolites in bile. Total radioactivity in each bile sample was measured by solubilizing 20 μl bile with 0.2 ml NCS, adding 10 ml Econofluor to this mixture, and determining dpm as described previously. For specific quantification of RA and retinoyl-β-glucuronide, 50 μl of each bile sample was injected directly on a reverse-phase column. Chromatographic conditions were the same as described for plasma except that the acetonitrile concentration in the mobile phase was decreased to 45% (v/v) and eluate fractions were collected at 0.5-min intervals. The absolute amounts of RA and its glucuronide were calculated from the radioactivity associated with the respective peaks and the specific activity of the initial dose.

Measurement of radioactivity in urine and feces. The 24-hr accumulation of feces from each rat was homogenized in 30 ml of an ascorbic acid–trisodium EDTA solution (0.5 mg/ml each, in water). Aliquots of fecal homogenate were placed on cellulose pads in small paper cups (Combusto-pads and Combusto-cones, Packard Instrument Co., Downers Grove, IL), treated with Combustaid (Packard Instrument Co.) and combusted in a Packard model B306 Tri-Carb Tissue Oxidizer. Tritiated water was collected in 15 ml of Monophase-40 scintillation mixture (Packard Instrument Co.). Disintegrations per minute were corrected for reproducible losses (9 per cent) during combustion. The amounts of radioactivity in urine samples and cage rinses were similarly quantified.

RESULTS

Assay for RA in plasma. The h.p.l.c.–u.v. absorbance assay for RA (83–50,000 pmoles/ml) in plasma generates a linear standard curve that exhibits an extraction efficiency for RA of 76 per cent when compared with the detector response to on-column standards. Extraction efficiency is somewhat higher (78–83 per cent) for lower concentrations (1–35 pmoles/ml) as shown by extraction of high specific activity [10-^{3}H]RA from plasma and subsequent determination of dpm in the methanol extract. Although quantification of RA can be accomplished using either the u.v. absorbance or the radiolabel assay, only the results of the radiolabel method are reported for the studies on RA disposition in rats. Correlation between the two assay techniques is good at RA concentrations in plasma of more than 83 pmoles/ml, but the radiolabel assay offers greater sensitivity with little or no compromise in specificity.

Endogenous RA in rat plasma cannot be detected using the above extraction, chromatographic and u.v. detection methods. Therefore, normal, physiologic levels of RA in rat plasma must be lower than 33 pmoles/ml (10 ng/ml), which is the lower limit of detection by the u.v. assay.

Kinetics of RA and of RA metabolites in plasma. The concentration of RA in rat plasma after i.v. infusions of RA (0.15, 0.25 and 5 mg/kg) is shown in Fig. 1. Each curve can be divided into three phases. An initial, rapid decline appears to be dose-dependent in that the slope becomes progressively steeper as the dose is diminished. A secondary phase exhibits a slower rate of decline and lasts substantially longer after a 5 mg/kg dose than after a 0.015 mg/kg dose. The general slope of this secondary phase is clearly dose-dependent, since the time required to halve the plasma concentration 30 min after administration of 0.015, 0.25 and 5 mg/kg is 40, 65 and 120 min respectively. At later times the slopes of the curves for all three doses become progressively steeper, demonstrating an obvious deviation from first-order kinetics. The elimination half-life of RA at later times is estimated to be about 19 min. These dose-dependent kinetics are not due to enterohepatic

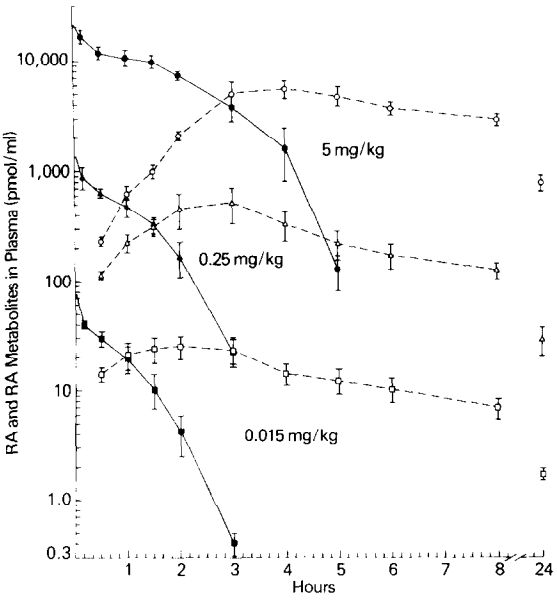

Fig. 1. Concentration of all-*trans*-retinoic acid (RA) (———) and its metabolites (– – – –) in rat plasma after an i.v. dose of [10-^{3}H]RA [17.3 μCi; 0.015 (□, ■), 0.25 (△, ▲) or 5 mg/kg (○, ●)]. A radiolabel assay that distinguishes between RA and its metabolites was used to specifically measure RA. Lower limits of detection decreased with decreasing dose, since the amount of radioisotope per rat remained constant while the dose decreased. Metabolite levels were determined by subtracting the concentration of RA from the concentration of total radiolabeled compounds in plasma. Each point and bar is the mean ± S.D. for three rats. For visual clarity, points and bars were not included at 2 and 5 min.

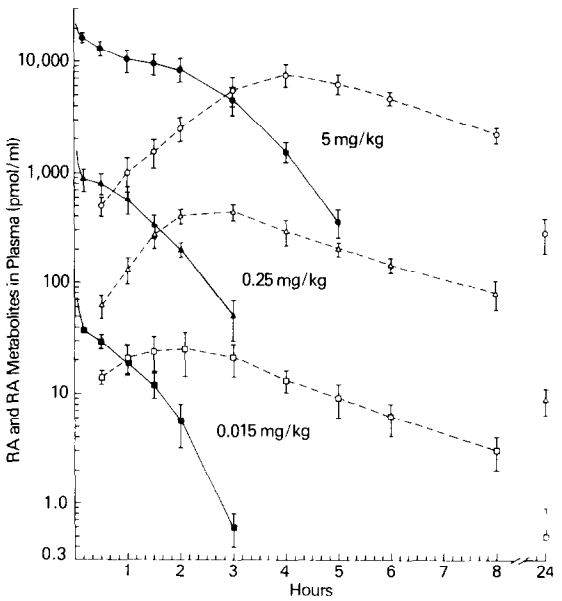

Fig. 2. Concentration of all-*trans*-retinoic acid (RA) (———) and its metabolites (– – – –) in plasma of rats with biliary fistulas after an i.v. dose of [10^{3}H]RA [18.5 μCi; 0.015 (□, ■), 0.25 (△, ▲) or 5 mg/kg (○, ●)]. A radiolabel assay was used to specifically measure RA. Metabolite levels were determined by subtracting the concentration of RA from the concentration of total radiolabeled compounds in plasma. Each point and bar is the mean ± S.D. for three rats. For visual clarity, points and bars were not included at 2 and 5 min.

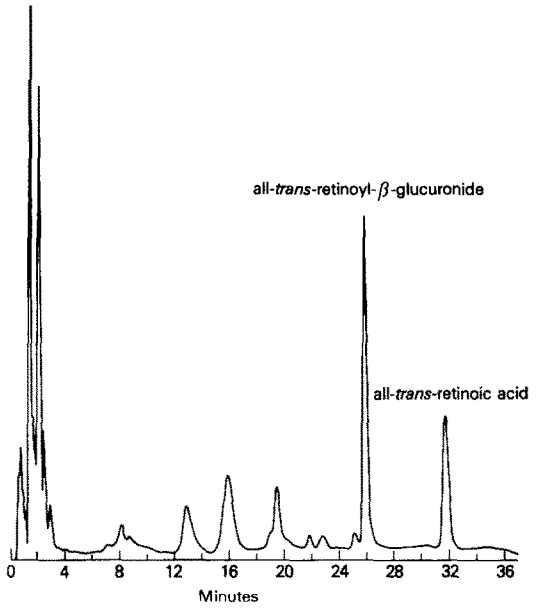

Fig. 3. Chromatogram of rat bile obtained 0–1 hr after 5 mg/kg all-*trans*-retinoic acid i.v. Chromatography was performed using detection at 365 nm on a Spherisorb 5 μm ODS reverse-phase column (temperature, 28°; flow, 2 ml/min) and an acetonitrile/1% ammonium acetate gradient. The effective concentration of acetonitrile was 25% for the first 13 min; it increased linearly for 15 min to 55% and then was maintained at 55%.

recirculation of RA, since the same kinetics were observed in rats with biliary fistulas (Fig. 2). Thus, interruption of the enterohepatic circulation did not cause a diminution in circulating levels of RA; on the contrary, RA levels were somewhat higher at later times after the two highest doses in rats with biliary fistulas. The latter phenomenon is probably not due to surgical stress, since one rat undergoing mock surgery (abdominal incision and bile duct isolation but no biliary catheterization) had the same circulating levels of RA (after 5 mg/kg RA, i.v.) as rats undergoing no abdominal surgery.

Figures 1 and 2 also show the circulating levels of radiolabeled RA metabolites after i.v. [10-^{3}H] RA. In contrast to RA, the metabolites of RA were not rapidly cleared from plasma and were significantly diminished in concentration by interruption of the enterohepatic circulation (at 24 hr, $P < 0.05$ by Student's *t*-test). The time at which metabolite levels achieved maximum values in plasma was dose-dependent. Thus, maximal levels occurred at 2 hr after 0.015 mg/kg RA, at 3 hr after 0.25 mg/kg, and at 4 hr after 5 mg/kg.

Identification of RAG. Figure 3 shows a chromatogram of bile obtained from a rat between 0 and 1 hr after an i.v. dose of RA (5 mg/kg). The substance that eluted at a retention time of 26 min was found to exhibit a u.v. spectrum essentially identical to that reported by Dunagin *et al.* [7] for RAG, i.e. a single absorbance maximum at 361 nm. Methylation of the metabolite with diazomethane generated a substance that possessed a mass spectrum (Fig. 4) consistent with monomethylated retinoyl-β-glucuronide. Thus, the spectrum reveals a molecular ion at *m/e* 490 and ions at *m/e* 472 and 458, corresponding to the loss of water and methanol, respectively, from the molecular ion. There are several ions in the spectrum (*m/e* 300, 285, 267, 256, and 255) that typically appear in the spectrum of RA also [24]. On the other hand, the base-catalyzed methylation procedure apparently hydrolyzed the metabolite, since the principle product has chromatographic and mass spectral properties identical to that of synthetic methylated RA. This observation is consistent with previous reports that *O*-ester glucuronides hydrolyze in the presence of chemical bases [25]. Finally, incubation of the metabolite with β-glucuronidase resulted in virtual disappearance of the metabolite and concurrent formation of RA. All of the above data indicate that the metabolite isolated from rat bile was, in fact, RAG.

Kinetics of RA and its metabolites in bile. The cumulative excretion of radiolabeled material into bile after i.v. injections of [10-^{3}H]RA is shown in Fig. 5. Although the total percentage of dose excreted within 24 hr is not statistically different for the two doses, there was a significant delay in excre-

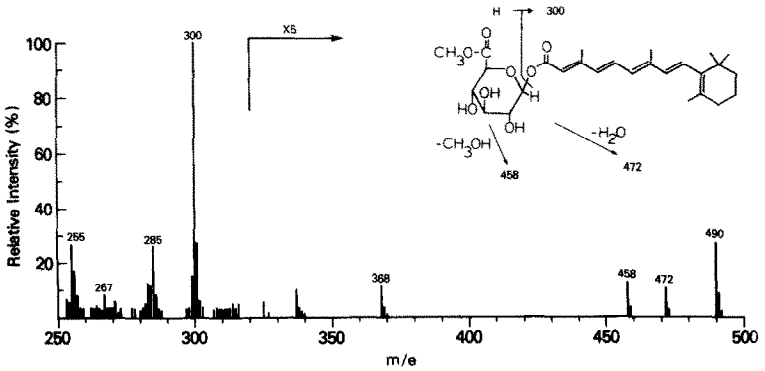

Fig. 4. Mass spectrum of methylated all-*trans*-retinoyl-β-glucuronide. The glucuronide was isolated from rat bile after an i.v. dose of RA (5 mg/kg) and then derivatized with diazomethane. Mass spectrometry was run in the electron impact mode at 70 eV ionizing voltage using a direct inlet probe at 200° and source temperature of 300°.

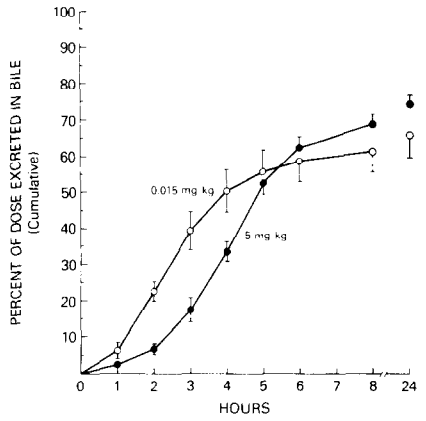

Fig. 5. Cumulative excretion of radiolabeled material into rat bile after i.v. injections of all-*trans*-[10-^{3}H]retinoic acid (18.5 μCi; 0.015 or 5 mg/kg).

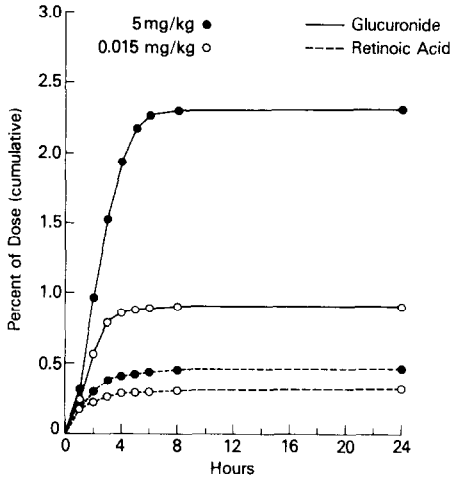

Fig. 6. Cumulative excretion of all-*trans*-retinoyl-β-glucuronide and all-*trans*-retinoic acid into rat bile after i.v. injections of [10-^{3}H]RA (18.5 μCi; 0.015 or 5 mg/kg). See text for details on quantification methods.

tion after the larger dose ($P < 0.05$ at 1, 2, 3, and 4 hr, Students's *t*-test). This suggests that the process of biliary excretion of RA and its metabolites into bile can be saturated by high levels of RA substrate.

The biliary kinetics of RA and RAG are shown in Fig. 6. The proportion of the dose excreted as intact RA was not significantly different for the two doses. Thus, for the range of doses examined, excretion of RA into bile was directly proportional to the dose level. On the other hand, the proportion of the dose converted to RAG increased with increasing dose. This same phenomenon has been noted for some xenobiotics, for example acetaminophen [12]. As indicated by Figs. 1 and 5, the shunting of excess RA into the glucuronidation pathway may result from saturation of other routes of metabolism which exhibit lower K_m values for RA than does glucuronyl transferase.

Excretion of [10-^{3}H]*RA in urine, feces, and bile.* Table 1 provides a comparison between high and low doses of RA with respect to gross excretion of [10-^{3}H]RA into urine, feces, and bile. The only notable dose-dependent alteration in the excretion pattern is the relatively greater importance of urinary excretion after lower doses. In addition, substantial evidence exists for an enterohepatic circulation of RA metabolites. First, a greater proportion of a dose was in the bile of catheterized rats than in the feces of normal rats. Thus, the percentage of biliary-excreted radiolabel that was subsequently reabsorbed was at least 26 and 34%, respectively, for the low and high doses. Second, interruption of the enterohepatic circulation caused a significant reduction in the amount of label excreted into urine. Therefore, approximately 25 per cent of the RA metabolites in urine must have been derived from biliary metabolites. These observations correlate well with the information in Figs. 1 and 2, which show that circulating levels of RA metabolites were reduced by catheterization of the bile duct.

DISCUSSION

Considerable evidence has been presented for dose-dependent kinetics of RA in rodents. Wang *et al.* [26] first observed non-first-order kinetics for RA (10 mg/kg, i.v.) in mice. We have now demonstrated in rats an obvious deviation from first-order kinetics for RA in plasma after various i.v. doses (0.015, 0.25, and 5 mg/kg). The degree of deviation from first-order kinetics can be diminished by lowering the dose. These observations are directly analogous to those made by Gerber and Arnold [15] for phenytoin in mice. In contrast to phenytoin and other xenobiotics, however, RA exhibits saturation kinetics at exceedingly low doses. For example, plasma levels of phenytoin did not demonstrate saturation kinetics until the dose surpassed 4–5 mg/kg, i.v. In contrast, plasma levels of RA showed non-first-order

Table 1. Excretion of total radiolabeled material within 24 hr after intravenous all-*trans*-[10-^{3}H]retinoic acid*

	Dose (mg/kg)	% Dose in urine	% Dose in feces	% Dose in bile	Total
Normal rats	0.015	30.5 ± 3.1†	48.3 ± 5.5‡		78.8 ± 5.9§
	5	19.8 ± 1.2‖	49.3 ± 6.2¶		69.1 ± 6.4**
Rats with biliary fistulas	0.015	22.0 ± 2.9††	1.7 ± 0.1‡‡	65.7 ± 6.2	89.4 ± 3.5§§
	5	15.4 ± 0.8‖‖	2.0 ± 1.0¶¶	74.3 ± 1.8	91.7 ± 4.3***

* Significant differences were established by Student's *t*-test ($P < 0.05$). † different from ‖, ††, ‖‖; ‖ different from †, ‖‖; †† different from †, ‖‖; ‡ different from ‡‡, ¶¶; ¶ different from ‡‡, ¶¶; § different from §§, ***; and ** different from §§, ***.

kinetics even at 0.015 mg/kg, i.v. There may be a dose of RA which, if sufficiently small, will result in a first-order decline in plasma. However, this may never occur since, at extremely low doses of RA, endogenous RA may constitute a significant proportion of total circulating RA. In addition to dose-dependent changes in plasma kinetics, it has been found that increases in dose levels result in: (1) a delay in achieving peak levels of metabolites in plasma, (2) a delay in biliary excretion of total metabolites, (3) a proportionally greater conversion of RA to RAG, and (4) a proportionally lesser excretion of RA metabolites into urine.

Using an assay that is specific for RAG, it has been demonstrated that only a small percentage of a dose of RA is excreted into rat bile as this compound. Since the proportion of RA converted to RAG diminishes with decreasing dose, it is concluded that glucuronidation must be a trivial metabolic pathway for the minute quantities of RA that are normally present *in vivo*. Unfortunately, this glucuronide has been repeatedly cited in the past as a major biliary metabolite of RA [6, 9, 27]. The source of discrepancy between current and past assertions is probably three-fold. First, data from former publications have been misconstrued, since the experimental results in these investigations have never proven that RAG constitutes a major portion of total RA metabolites. Specifically, Dunagin *et al.* [7] reported that 45 per cent of a dose of [6,7-^{14}C]RA was excreted in rat bile within 8 hr, that 50 per cent of the biliary metabolites of RA cochromatographed as polar, acidic compounds, and that RAG represented about 45 per cent of this polar material. Since only a small amount of RAG would be excreted after 8 hr (see Fig. 6), the total proportion of the dose excreted in bile as this metabolite would be on the order of 10–11 per cent. A second reason for previous overstatement of the importance of the glucuronidation reaction could be the poor resolution that is inherently characteristic of former liquid chromatographic procedures. Thus, contamination of the RAG peak with other radiolabeled metabolites could result in over-estimation of the amount of this glucuronide. Finally, in the work cited above, massive doses of RA (4 mg or approximately 18 mg/kg) were infused directly into the liver via the portal vein. This method of dose administration could result in a proportionately greater conversion of RA to RAG, since our data show that the percent conversion of RA to its *O*-ester glucuronide increases with increasing dose. A 10 per cent conversion of RA to RAG is feasible under these conditions; however, this pattern of metabolism probably bears little resemblance to that found for physiological quantities of RA.

Although some metabolic pathways for RA have been elucidated, it is still not possible to determine which pathways are quantitatively the most important. After i.v. administration of approximately 0.044 mg/kg RA, Roberts and DeLuca [28] observed that about one-third of the RA is decarboxylated in the rat. In contrast, Hänni *et al.* [10] and Hänni and Bigler [6] found that decarboxylation is only a minor route of RA metabolism. The latter workers, however, employed relatively enormous doses of RA (approximately 68 mg/kg, i.p.), which could alter the relative abundance of various metabolites. Recent studies have demonstrated *in vitro* that the C-4 position of RA can be sequentially oxidized to generate 4-hydroxy- and 4-oxoretinoic acids [29]. The quantitative prevalence of this pathway has not been determined *in vivo*, although Hänni and Bigler [6] have shown that 4 per cent of a large dose of RA appears as all-*trans*-4-oxoretinoic acid in rat feces. Two other fecal metabolites (all-*trans*-18-hydroxyretinoic acid and 9-*cis*-18-hydroxyretinoic acid) were also characterized, and these constituted 20 per cent of the dose.

In conclusion, future studies on RA disposition in animals should be designed to discern possible dose-dependent effects on experimental results. Without this precaution, correlations between the fate of exogenous RA and the fate of metabolically derived RA may be falsely generated.

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