A multicompartmental model of vitamin A kinetics in rats with marginal liver vitamin A stores

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Abstract A linear, first-order, constant-coefficient multicompartmental model is presented which describes the dynamics of [3H]retinol turnover in adult rats with normal plasma retinol concentrations but low liver stores (<100 μg of retinol equivalents). To fit plasma and tissue (liver, kidney, and rest of carcass) tracer and tracee data, eight physiological compartments were required in the model: two in plasma (proposed to correspond to the retinol transport complex, and retinyl esters in plasma lipoproteins) and two each in liver, kidneys, and other extrahepatic tissues. Extensive recycling of retinol among plasma, liver, and the rest of carcass was also required. The model predicted that 44% of whole body vitamin A (145 μg) was in extrahepatic tissues. The vitamin A utilization rate (system disposal rate) was 6.9 μg of retinol equivalents/day. The system residence time (mean sojourn time) for vitamin A was 21 days, and the fractional catabolic rate for the system was 5%. The mean transit time (turnover time) for vitamin A in its plasma retinol transport complex was 0.078 days (1.9 hr); the residence time was 0.98 day, versus 11 days in the liver, 9 days in carcass, and 0.54 days in kidneys. The model predicted that, of the plasma turnover, 48% recycled to the liver and 52% to extrahepatic tissues. The liver retinol secretion rate was 48 μg/day, more than half of which was from recycled plasma retinol. Since the plasma retinol turnover rate (87 μg/day) was 13 times the system disposal rate, the data suggest that this is a high response system in which changes in the dynamics of recycling of retinol allow for rapid adjustment in vitamin A distribution in response to changes in nutritional, metabolic, or physiological conditions; and in which plasma retinol levels are controlled homeokinetically by changes in hepatic and extrahepatic recycling of holo retinol-binding protein. — Green, M. H., L. Uhl, and J. B. Green. A multicompartmental model of vitamin A kinetics in rats with marginal liver vitamin A stores. J. Lipid Res. 1985. 26: 806–818.

Supplementary key words compartmental analysis • retinol turnover • vitamin A metabolism

Although vitamin A is involved in a number of critical functions in mammalian systems, many quantitative and descriptive features of the dynamics of its metabolism in vivo are not well defined. Specifically, our present understanding of vitamin A metabolism (1) is not sufficiently detailed to explain the homeostatic mechanisms that regulate plasma vitamin A concentrations at different levels of vitamin A nutrition, the dynamics of hepatic vitamin A storage, the regulation and mechanisms of retinol delivery to vitamin A-requiring tissues, or potential differences in vitamin A utilization accompanying changes in vitamin A status.

In recent years, Berman and colleagues (2-4) have made a significant contribution to the field of tracer kinetic analysis by developing and applying mathematical and computer methods for analyzing plasma and tissue turnover data by compartmental analysis. These methods have been used to develop mechanistic models describing present understanding of the metabolism of many bloodborne constituents (5-9). In this approach, data from an in vivo turnover study are analyzed in the light of an initial conceptual model which is compatible with a priori knowledge or assumptions about the system under study. The analysis generates a revised or expanded compartmental model that is consistent with current data and it quantitates the kinetic parameters defining the model. Confidence intervals for these parameters are also provided.

In the experiments reported here, we applied the techniques of multicompartmental tracer kinetic analysis to the study of vitamin A turnover in the rat. A nonperturbing dose of radiolabeled retinol in its physiological transport complex was administered to moderately vitamin A-sufficient rats. A mechanistic model of retinol turnover was generated by compartmental analysis of plasma and tissue kinetic data collected in short- and long-term studies. In addition to quantitating parameters describing the kinetics of retinol turnover, this analysis provides some new descriptive insights into the dynamics of vitamin A metabolism.

Abbreviations: HPLC, high performance liquid chromatography; ROH, retinol; RAc, retinyl acetate; RP, retinyl palmitate; RE, retinyl esters; REq, retinol equivalents; RBP, retinol-binding protein; TTR, transthyretin (formerly called prealbumin); FSD, fractional standard deviation; FCR, fractional catabolic rate; ST, short-term; LT, long-term.
METHODS

Animals and diets

Male Sprague Dawley rats (Hilltop Lab Animals, Scottdale, PA) were individually caged and provided with food and water ad libitum. Rats to be used as donors of plasma containing radioactive retinol were obtained as weanlings and fed a vitamin A-free purified diet containing (per 100 g): casein (18 g), methionine (0.23 g), dextrose (30.4 g), corn starch (33.2 g), peanut oil (10 g), mineral mix (3.5 g; AIN 76, Teklad Test Diets, Madison, WI), vitamins (2.3 g; modified from formulation in ref. 10), and agar (2.4 g) for 63 days. Rats to be used as recipients (n = 11) were obtained at a body weight of 90–100 g and were fed the vitamin A-free diet for 21 days. They were then fed a similar diet that contained 0.5 μg of retinol equivalents (REq) as retinyl palmitate per gram of diet (Sigma Chemical Co., St. Louis, MO) for the following 50 days and during the subsequent turnover studies. This regimen provided a daily intake of approximately 9 pg of REq, and was intended to maintain recipients in a steady state with respect to vitamin A.

Body weights and estimates of food intake were recorded twice weekly for all rats. Until the turnover study, blood samples (0.2 ml) were collected weekly between 0830 and 1030 hr from a caudal vein into tubes containing EDTA (final concentration = 4 μM). Aliquots of plasma were stored at −20°C under nitrogen atmosphere for subsequent determination of plasma retinol concentration.

Isotopes and chemicals

(11,12 (n)-3H)Vitamin A acetate (sp act 180 μCi/μg; Amersham Corp., Arlington Hts, IL) was used as purchased. All-trans-retinyl-11-3H]acetate (sp act 5.95 μCi/μg; SRI International, Menlo Park, CA) was a generous gift of the National Cancer Institute's Division of Cancer Cause and Prevention. Solvents were reagent- or HPLC-grade, as appropriate. Chemical standards of retinol (ROH), retinyl acetate (RAc), and retinyl palmitate (RP) for liquid chromatography were obtained from Sigma.

All procedures involving vitamin A were carried out under amber light.

Preparation of retinol-labeled plasma

A dispersion of 3H-labeled RAc was prepared for injection into donor rats using a modification of published procedures (11). Solvent was removed from a mixture of the two isotope preparations (about 500 μCi, 5–7 μg of REq) at 37°C using a gentle stream of nitrogen. The labeled compounds were resolubilized in 75 μl of absolute ethanol, and 100 μl of Tween 40 (Sigma) was added. The mixture was vortexed for 30 sec, during which time 225 μl of pyrogen-free sterile physiological sodium chloride solution and 225 μl of sterile water were added dropwise.

The dispersion was refrigerated overnight under nitrogen atmosphere and was injected within 18 hr of preparation. Eighty-seven percent of the label was found to elute with RAc when the dispersion was analyzed by reverse-phase high performance liquid chromatography (HPLC).

For preparation of labeled plasma, donor rats (plasma ROH, 2 and 5 μg/dl) were anesthetized with diethyl ether; the dispersion was injected into an exposed jugular vein. When plasma radioactivity was maximal (180 and 120 min after injection), donors were reanesthetized and blood was collected by closed chest cardiac puncture into syringes containing sodium citrate as anticoagulant. Plasma was harvested by low speed centrifugation at 4°C, and unmetabolized Tween micelles (5% of total plasma radioactivity in a similar preparation) were removed by ultracentrifugation (L8-70 ultracentrifuge, SW 41 rotor; Beckman Instruments, Palo Alto, CA) at the background density of plasma for 1.5 × 10^4 g-min. The resulting [3H]retinol/retinol-binding protein/transferrin-labeled plasma ([3H]-ROH/RBP/TTTR) was stored under nitrogen atmosphere at 4°C and was injected into recipients within 24 hr of collection.

To verify that the labeled retinol was carried in its physiological transport complex, we used methods similar to those described by Muto, Smith, and Goodman (12). An aliquot of labeled plasma (spiked with unlabeled rat plasma) was applied to a 2.5 × 50 cm column of Sephadex G-100 (Pharmacia Fine Chemicals, Piscataway, NJ). The column was eluted with 0.058 M phosphate buffer, pH 7.4. Fractions that eluted after the void volume were assayed for radioactivity; those containing significant amounts of radioactivity were analyzed for RBP concentration by radioimmunoassay (10).

In vivo turnover studies

Recipient rats were randomly divided into a short-term (ST; n = 6) or long-term group (LT; n = 5). For turnover studies, nonfasting rats were anesthetized with methoxyflurane (Pittman-Moore, Washington Crossing, NJ) between 0830 and 1000 hr; a blood sample was collected from a caudal vein for determination of plasma retinol concentration. Weighed aliquots of [3H]-ROH/RBP/TTTR-labeled plasma (0.8 g) were injected into an exposed jugular vein, and anesthesia was immediately removed. Serial blood samples (0.2 ml; n = 33 for LT animals, n = 14–21 for ST rats) were collected at geometrically increasing intervals from 3 min until the rats were killed. Aliquots of plasma were stored at −20°C under nitrogen atmosphere for subsequent analysis.

ST rats were killed 1 (n = 2), 2 (n = 2), or 15 days (n = 2) after dose administration; LT recipients were killed 35 days after injection. Rats were anesthetized with diethyl ether, and a large terminal blood sample was obtained by closed chest cardiac puncture; the rats were then
perfused through the thoracic aorta with 100 ml of phosphate-buffered saline, pH 7.4. Livers, kidneys (decapsulated), adrenals, small intestine (rinsed of contents), eyes, skin, and remaining carcass (stomach, cecum, and colon rinsed of contents) were isolated, weighed, frozen, freeze-dried, and stored at ~20°C.

**Plasma and tissue analyses**

Retinoids in aliquots of plasma (≤0.1 ml) were extracted (13, 14) into hexane (5 ml) from 40% aqueous ethanol (4 ml). An internal standard (retinyl acetate in ethanol) was added to all plasma samples before extraction. After vortexing and centrifugation, aliquots of the neutral lipid extract were taken for liquid scintillation spectrometry and/or HPLC. Butylated hydroxytoluene (5 µg/ml; Sigma) was added as an antioxidant to the latter samples.

Aliquots (0.15 g) of freeze-dried liver, kidneys, and small intestines, or whole freeze-dried tissues (eyes and adrenals) were reconstituted with water and then saponified (13) for 1 hr at 60°C in 20% ethanolic potassium hydroxide (1.5 ml) containing pyrogallol (0.7% w/w). The nonsaponifiable material, including retinol, was extracted into 5 ml of hexane after addition of 1 ml of water. The aqueous phase was washed twice with the same volume of hexane. Aliquots of the pooled extract were taken for HPLC analysis (livers and kidneys) and/or for counting as described. Skins and carcasses were saponified in 50% aqueous KOH (500 and 1000 ml, respectively) in an autoclave for 1 hr at 100°C, 18 psi. The saponified samples were filtered, and aliquots (7 ml) were extracted from 6 ml of 95% ethanol into 14 ml of hexane. After removal of hexane, the aqueous phase was washed twice with 14 ml of hexane. Aliquots of the pooled extracts were chilled to sediment a white flocculent contaminant. After removal of the sediment, aliquots of the extract were analyzed for radioactivity. Tissue radioactivity data are presented for liver, kidneys, and remaining carcass (sum of small intestine, eyes, adrenals, skin, and carcass).

For HPLC analysis, solvent was removed under nitrogen from lipid extracts and samples were solubilized in absolute ethanol. For plasma samples, retinol, retinyl acetate (internal standard), and retinyl esters (RE) were separated by HPLC (Model 6000A, Waters Associates, Milford, MA), using a 0.46 × 15 cm reverse-phase column (Ultrasphere ODS 5µ; Beckman Instruments, Berkeley, CA) and a mobile phase of methanol. Liver and kidney total retinol masses were analyzed under similar conditions. Retinoids were detected by UV absorbance at 340 nm (Model 441, Waters), and peak areas were determined by digital integration (Model 3390A; Hewlett Packard, Palo Alto, CA). Retinol masses, corrected for recovery of the internal standard in the case of plasma samples (average recovery = 95 ± 6%), were calculated by comparison to the mass: area ratios of pure chemical standards of retinol and retinyl acetate. These standards had been previously quantitated at 325 nm (Acta CIII, Beckman Instruments, Fullerton, CA) using the following extinction coefficients (ε₅₄₄₅) calculated from the data of Boldingh et al. (15): 1835 for ROH and 1560 for RAc. For radioactive plasma samples, retinol and retinyl ester eluates from the HPLC were collected directly into glass vials for counting.

Lipid extracts and HPLC eluates were counted (LS-8100, Beckman Instruments, Irvine, CA; SL-30, IN/US Service Corp., Fairfield, NJ; or Model 3385, Packard Instruments, LaGrange, IL) in a toluene-based scintillation solution containing 0.4% 2,5-diphenyloxazole (PPO). Samples were counted twice either to a 2-sigma error of 2.0% or less, or for 180 min. Only plasma RE fractions required the latter condition. Quenching, as monitored by external standardization, was negligible; cpm were converted to dpm using [³H]toluene standards (Amersham).

**Statistical and kinetic analyses**

Data on pre- and post-turnover plasma retinol concentrations were analyzed by the Statistical Analysis Systems general linear models program (16).

Data on plasma and tissue tracer and tracee concentrations versus time were analyzed by compartmental analysis using the SAAM 27 (Simulation, Analysis and Modeling; 3) and CONSAM programs (Conversational SAAM; 4) run on a VAX 11/780 computer (Digital Equipment Corp., Marlboro, MA). Individual animal data on plasma ROH and RE tracer concentrations at each time for the five rats killed 35 days after dosing were first normalized to the estimated plasma tracer concentration at the time of injection, t₀ (t₀ dpm/ml = dpm injected / estimated plasma volume [ = body weight (g) × 0.04; 17]). Data were then arithmetically averaged to obtain the group mean data. Based on the group variance for plasma retinol tracer data, a fractional standard deviation (FSD = SD/mean) of 0.05 was used in the kinetic analysis. Due to the greater measurement error resulting from the low count rate in plasma RE, an FSD of 0.15 was assigned. Normalized plasma ROH tracer concentrations were also calculated for the ST groups. These latter data were plotted and qualitatively examined to verify that plasma responses were kinetically similar to the LT group; however, they were not used for modeling. Fraction of the injected dose recovered in tissues or irreversibly lost (loss = 1 – fraction recovered in plasma + tissues) was also calculated for each group. Tissue tracer data from the ST recipients were used in the modeling process to define the early dynamics of tissue retinol turnover. An FSD of 0.05 was assigned to LT tissue and loss data, and a value of 0.10 to ST tissue and loss data.

Group mean plasma and tissue tracer and tracee data,
and information on tracer loss, were used simultaneously for model identification. To develop the mechanistic model presented here, a simple model for vitamin A metabolism was first conceptualized. Initial estimates of model parameters and the model itself were then adjusted using the interactive CONSAM program to obtain a consistent, best-fit of mathematically simulated plasma, tissue and loss data to the observed values (i.e., residuals randomly distributed and minimized). Final parameter values and estimates of the fractional standard deviation for each adjustable parameter were then generated using SAAM’s iterative, nonlinear least squares routine.

A method of estimating maximum likelihood, the weighted version of Akaike’s Information Criterion (AIC; 18) was used to a) identify the “best” model when compatible alternate models were being evaluated and b) statistically test whether increases in model complexity were justified (i.e., significantly decreased the residual sum of squares in spite of an increased number of parameters):

$$AIC_p = L + 2p$$

where $p$ = number of adjustable parameters

$$L = n \ln RSS - \frac{p}{2} \ln W_i$$

$n$ = number of data points

$$RSS = \text{weighted sum of squares of residuals} = \sum_i W_i (QO_i - QC_i)^2$$

Wi (normalized weight) = 1/(SD_i)$^2$

$$\sum W_i = n.$$ For a model to be considered statistically “better” than others, it must reduce $AIC_p$ by more than 1-2 units.

**Model-based calculations**

Nomenclature and method of calculation for kinetic parameters is similar to that used by others (9, 19, 20). Parameters reported include:

- Rate constants, $L(I,J)$’s, or the fraction of compartment $J$’s mass, $M(J)$, transferred to compartment $I$ per unit time.
- Fractional catabolic rates, $FCR(I,J)$, or the fraction of particles in compartment $I$ which leave irreversibly per unit time after introduction into the system via compartment $J$: $U(J)$.
- Mean transit times, $i(I)$, or the time an average vitamin A molecule spends in compartment $I$ during a single transit; $i(I) = 1 \sum_{J=1}^{N} L(I,J) = 1/L(I,I)$.
- The mean residence time, $\bar{T}(I,J)$, or the total time an average vitamin A molecule spends in compartment $I$ before irreversible loss following its introduction into the system via compartment $J$: $\bar{T}(I,J) = 1/FCR(I,J)$.
- The mean number of recyclings, $r(I)$, through average vitamin A molecule recycles to compartment $I$ before irreversible loss: $r(I) = [\bar{T}(I)/i(I)] - 1$.
- Mean recycling time, $\bar{t}(I,I)$, or the time it takes for an average vitamin A molecule to recycle to compartment $I$ following departure from that compartment: $\bar{t}(I,I) = [\bar{T}(SYS) - \bar{T}(I(I))/r(I)]$.

In addition, using model-predicted compartment masses, $M(I)$, the system disposal rate, $DR(SYS) = FCR(I,J) \times M(I)$ (which equals the system production [$U(SYS)$] in a steady state), and transfer rates, $R(I,J) = L(I,J) \times M(J)$, were calculated.

**RESULTS**

Body weights of recipient rats at the beginning of the turnover studies averaged 422 ± 32 g ($N = 11$). After a slight weight loss during the first 72 hr of the LT turnover study (2.5%), rats maintained or slightly gained body weight. Body weights averaged 437 g during the LT turnover study and 454 ± 11 g ($n = 5$) at the end of the 35-day experiment.

Upon arrival, plasma retinol concentration of recipients averaged 59 ± 4 $\mu$g/dl ($n = 10$). Retinol levels averaged 51 ± 5 $\mu$g/dl ($n = 10$) at the initiation of the turnover experiments, 43 ± 7 $\mu$g/dl ($n = 5$) 17 days after injection, and 44 ± 5 $\mu$g/dl ($n = 5$) in rats killed 35 days after dosing. The mean plasma ROH concentration during the turnover study for the LT group was 46.1 ± 3.7 $\mu$g/dl. This was significantly lower than during the pretreatment period ($F_{1,10} = 11.76; P < 0.006$) and may be related to the stress of repeated blood collections. To evaluate the effect of the frequent blood samplings at the beginning of the turnover study, we measured hematocrits immediately before dose injection and 4.5 hr later in the LT group (after seven blood samples had been taken). Initial average hematocrit (45 ± 4%) was not significantly different from that measured 4.5 hr later (48 ± 2%).

Total liver vitamin A levels averaged 82 ± 30 $\mu$g for the four rats killed 1 and 2 days after dose administration, 48 ± 4 $\mu$g for the two killed at 15 days, and 43 ± 16 $\mu$g for those killed at 35 days. Thus, recipients appeared to be in...
a slightly negative hepatic vitamin A balance (about 2.5 μg/day) during the first 2 weeks of the turnover studies. Total kidney vitamin A levels averaged 3.4 ± 0.9 μg for the four rats killed 1 and 2 days after dosing and 2.8 ± 0.4 μg for the LT group.

[^H]Retinol-labeled plasma

Vitamin A-depleting rats were used for preparation of[^H]retinol-labeled plasma in an attempt to maximize the mass and specific activity of[^H]-ROH/RBP secreted from the liver (10). Estimated recovery of injected radioactivity in the total plasma volume was less than 25% for both donors.

The similar elution profiles for radioactivity and RBP in one of the preparations of labeled plasma are shown in Fig. 1. Ninety percent of the assayed radioactivity coincided with the RBP peak. In addition, RBP elution volume corresponded to an elution volume for proteins of approximately 70,000 daltons, the molecular weight of the physiological transport complex. Based on these observations, we conclude that the labeled plasma used for in vivo turnover studies contained mainly[^H]retinol complexed to RBP and TTR.

The labeled plasma aliquots administered to rats killed 1, 2, and 15 days after injection contained 86.7 ± 0.4 ng retinol (3.5 μCi), and those injected into rats in the long-term group, 156.7 ± 0.4 ng (8 μCi). These doses thus caused an average perturbation of 1.1 ± 0.2 and 1.8 ± 0.2%, respectively, of the recipients' estimated plasma vitamin A pool.

Model development and interpretation

Group average data on relative plasma ROH and RE tracer concentrations versus time after dosing are shown in Fig. 2 for rats killed 35 days after injection of[^H]-ROH/RBP/TTR-containing plasma. Plasma radioactivity
declined rapidly during the first 75 hr after dose administration, and then more gradually. Recovery of hexane-extractable radioactivity in saponified tissues is presented in Table 1. The maximum recovery of radioactivity in the liver was 36% of the injected dose at 2 days after dose administration. At 35 days, 6% of the dose was recovered in the liver. Recovery of carcass radioactivity declined from an observed maximum of 26% 2 days after dosing, to 8% at 35 days. By the end of the experiment, an average of 15 ± 4% of the injected dose was recovered in the animals.

We began the modeling process by postulating a simple

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**TABLE 1. Observed and model-predicted tissue radioactivity**

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver</th>
<th>Kidneys</th>
<th>Carcass</th>
<th>Output</th>
<th>Fraction of Injected Dose</th>
<th>Output</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QO</td>
<td>QC</td>
<td>QO</td>
<td>QC</td>
<td>QO</td>
<td>QC</td>
</tr>
<tr>
<td>1 Day</td>
<td>0.290</td>
<td>0.310</td>
<td>0.0367</td>
<td>0.0348</td>
<td>0.242</td>
<td>0.244</td>
</tr>
<tr>
<td></td>
<td>0.285</td>
<td></td>
<td>0.0456</td>
<td></td>
<td>0.284</td>
<td>0.305</td>
</tr>
<tr>
<td>2 Days</td>
<td>0.370</td>
<td>0.340</td>
<td>0.0116</td>
<td>0.0252</td>
<td>0.204</td>
<td>0.199</td>
</tr>
<tr>
<td></td>
<td>0.350</td>
<td></td>
<td>0.0310</td>
<td></td>
<td>0.219</td>
<td>0.361</td>
</tr>
<tr>
<td>15 Days</td>
<td>0.145</td>
<td>0.162</td>
<td>0.0156</td>
<td>0.00839</td>
<td>0.107</td>
<td>0.153</td>
</tr>
<tr>
<td></td>
<td>0.142</td>
<td></td>
<td>0.00824</td>
<td></td>
<td>0.118</td>
<td>0.716</td>
</tr>
<tr>
<td>35 Days</td>
<td>0.0604</td>
<td>0.0635</td>
<td>0.00327</td>
<td>0.00338</td>
<td>0.0810</td>
<td>0.0754</td>
</tr>
<tr>
<td></td>
<td>(0.0262)</td>
<td></td>
<td>(0.00073)</td>
<td></td>
<td>(0.0200)</td>
<td>(0.0421)</td>
</tr>
</tbody>
</table>

*Data are observed (QO) and model-predicted (QC) fractions of injected radioactivity recovered in tissues or irreversibly lost (output). Individual values of QO are presented for rats killed 1, 2, and 15 days after dosing; QO for rats killed at 35 days is mean (SD) (n = 5).

*Output = 1 - [fraction of dose in plasma + liver + kidneys + rest of carcass].

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conceptual model for retinol metabolism which was compatible with currently accepted ideas on vitamin A turnover (1). In the initial five-compartment model, chylomicron retinyl esters were delivered to the liver; and retinol in its physiological transport complex was secreted into plasma by the liver and delivered to tissues (kidney vs rest of carcass). The model incorporated irreversible loss of retinol after utilization by tissues and recycling of retinol to the plasma and from plasma to the liver. Current group average plasma ROH, plasma RE, and tissue tracer data could not be fit to this model. One reason was that these data were not compatible with time-invariant rate constants for irreversible loss of tracer from tissues. We hypothesized that this might be due to a nonphysiological component in the dose, and thus introduced a plasma compartment (compartment 2, not shown in the final model) that contained negligible mass and was rapidly cleared. Inclusion of this compartment was necessary to approximate data on irreversible disposal of tracer in the rats killed at 1 and 2 versus 15 and 35 days. Secondly, the fit was significantly improved when we included two kinetically distinct pools of vitamin A in the liver, kidneys, and rest of carcass (AIC<sub>17</sub> = -709.3 vs AIC<sub>13</sub> = -613.0 for the model with one compartment/tissue). The resulting mechanistic model is shown in Fig. 3; tentative physiological correspondence of model components is included in the following description of the final model.

Retinol associated with the plasma RBP/TTR complex was modeled as compartment 11. Compartment 21, which contained 2–5% of total plasma retinoid radioactivity, is proposed to represent RE in plasma lipoproteins (21, 22). In order to fit early time points, the data required that 0.14% of the dose was initially in compartment 21. The liver, kidney, and rest of carcass each required one smaller, fast-turning-over compartment (compartments 3, 13, and 16, respectively), and one larger, more slowly turning-over compartment (compartments 5, 14, and 17). System input of absorbed dietary vitamin A was into the liver (compartment 3). Extensive recycling of retinol among plasma, liver, and extrahepatic tissues was required.

In several cases, when no a priori quantitative information was available, assumptions were made during the modeling process that established dependent relationships among certain model parameters without violating known

Fig. 3. Proposed model for vitamin A turnover. Compartments are represented by circles; large triangles are summer functions; small ones indicate sites of tissue sampling. Data shown are model-derived rate constants, $k(I,J)$ ± (estimated fractional standard deviation); that is, the fraction of compartment $J$'s mass transferred to compartment $I$ time (day)$^{-1}$. Rate constants were generated by simultaneously modeling long-term pup's plasma data, and all tissue data. IC(1) is the calculated fraction of the dose in compartment 1 at 0. IC(11) = 0.79; IC(21) = 0.0014. K(15), the model-derived correction for the initial volume of distribution of the dose, equals 1.82. Plasma compartment 2, the nonphysiological component of the dose, is not shown. The model predicted that it initially contained 20.7% of the dose and had an FCR of 50.3 per day.
characteristics of the system. This was done to minimize the number of parameters in the model. First, compartments 3, 13, and 16, the smaller, rapidly turning-over tissue compartments, were designated as sites of irreversible loss of tracer, with \( L(0.3) = L(0.13) = L(0.16) \). "Irreversible loss" includes all elimination processes, both degradative and excretory. Also, \( L(11.16) = L(11.13), L(17.16) = L(14.13), \) and \( L(16.17) = L(13.14) \). Finally, it was assumed that rate constants were time-invariant. These assumptions will be discussed later.

Compatibility of the data with the final model shown in Fig. 3 is evident by comparing both the fit of the simulated data to observed plasma tracer concentrations (Fig. 2) and from the agreement between model-predicted and observed values for tissue recovery of tracer (Table 1). The mean fractional standard deviation generated by SAAM's nonlinear regression analysis (3) for observed versus model-predicted plasma \(^3\)H-labeled ROH (compartments 11 + 2) was 0.087; for plasma RE, it was 0.090. The mean fractional standard deviations for observed versus calculated data were 0.052 for liver, 0.039 for kidney, 0.095 for carcass, and 0.095 for the irreversible loss sink.

Model-derived kinetic parameters

Rate constants \([L(I,J)]'s\) for the final model are shown in Fig. 3; kinetic parameters derived from them are presented in Table 2. An average vitamin A molecule spent 0.078 day (1.9 hr) in the plasma (compartment 11) during a single transit. The fractional catabolic rate for compartment 11 with system input into compartment 3 [FCR(11,3)] was 1.015/day. Thus, plasma residence time was 0.98 day. An average vitamin A molecule recycled through plasma 11 times and was sequestered outside the vascular compartment for 1.7 days (recycling time).

The mean transit time for vitamin A in the fast turning-over liver compartment (compartment 3) was 0.024 day (0.57 hr) versus 3 days in the slowly turning-over compartment (compartment 5). For the carcass, the mean transit time was 0.26 day (6.3 hr) in the rapidly turning-over compartment (compartment 16) versus 9 days in compartment 17. The mean residence time for vitamin A in the liver was 10.6 days; in carcass, 8.6 days; and in kidneys, 0.54 day. An average vitamin A molecule recycled nine times through the rapidly turning-over liver pool (compartment 3), two times through compartment 5, and five times through the fast turning-over carcass compartment (compartment 16). An average molecule did not recycle through compartments 13, 14, 17, or 21.

The system residence time for a typical vitamin A molecule (as ROH, RE or retinaldehyde) was 20.7 days. That is, after entering compartment 3, an average molecule remained in the system almost 3 weeks before irreversible loss due to degradation or excretion. The system FCR was 0.048/day; thus, 5% of the animals' vitamin A was irreversibly lost each day.

In order to calculate several other parameters of interest, the mass of plasma compartment 11 was estimated \([\text{= mean LT plasma ROH concentration (pg/ml)} \times \text{estimated plasma volume (ml)} / K(15)], \text{where K(15) is the model-derived correction for the estimated initial volume of distribution of the dose (=} 1.182)] \). The system disposal rate \([\text{DR(SYS)}] = \text{FCR}(11,3) \times \text{M(11)}] was 6.9 \mu\text{g REq/day}. Then, using the disposal rate as an estimate of the time-weighted mean input rate \([U(3)]\), we calculated other compartment masses and transfer rates \([R(I,J)]'s\; \text{Fig. 4}]); assuming a tracee steady state. (If rats were actually in a slight negative balance at the beginning of the turnover study, model-predicted values represent a time-weighted mean for compartment masses.) The total traced mass was 143 \mu\text{g of REq}. Surprisingly, the model predicted significant amounts of vitamin A in extrahaepatic tissues in these rats with marginal vitamin A status (44% of the total traced mass, vs 5% in plasma and 51% in liver). The model predicted that 98% of total liver vitamin A was in the slowly turning-over pool (compartment 5), and that 78% of the vitamin A in extrahaepatic tissues was in slowly turning-over pools (compartments 17 + 14).

The plasma ROH turnover rate \([\text{=} R(3,11) + R(16,11) + R(13,11) + R(21,11)]) was 87 \mu\text{g/day}. Thus, compared to the system disposal rate, only 8% of the total plasma ROH turnover was irreversibly lost (vs recycled) in these rats. Of this plasma ROH turnover, 41 \mu\text{g/day (48%)} recycled to the liver and 52% transferred to extrahaepatic tissues. Of the total input into plasma, 48 \mu\text{g/day (55%)} was due to hepatic input of holoRBP, and 45% represented recycling from extrahaepatic tissues. Of the liver input into the plasma ROH pool, 56% came from recycling of plasma holoRBP \([R(3,11)/R(3,3)], 1% \text{from recycling of plasma RE} [R(3,21)/R(3,3)], 33% \text{from the slowly turning-over hepatic compartment} [R(3,5)/R(3,3)], \) and 9% from dietary input \([U(3)/R(3,3)]]\). Of the non-irreversi-

### Table 2. Model-derived kinetic parameters

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Transit Time *</th>
<th>Residence Time *</th>
<th>Recycling Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 (Plasma ROH)</td>
<td>0.078 days</td>
<td>0.98 days</td>
<td>11.6</td>
</tr>
<tr>
<td>21 (Plasma RE)</td>
<td>0.21 days</td>
<td>0.021 days</td>
<td>0</td>
</tr>
<tr>
<td>3 (Liver)</td>
<td>0.024 days</td>
<td>0.25 days</td>
<td>9.6</td>
</tr>
<tr>
<td>5 (Liver)</td>
<td>2.9 days</td>
<td>10.3 days</td>
<td>2.5</td>
</tr>
<tr>
<td>13 (Kidney)</td>
<td>0.26 days</td>
<td>0.23 days</td>
<td>0</td>
</tr>
<tr>
<td>14 (Kidney)</td>
<td>3.0 days</td>
<td>0.31 days</td>
<td>0</td>
</tr>
<tr>
<td>16 (Carcass)</td>
<td>0.26 days</td>
<td>1.7 days</td>
<td>5.3</td>
</tr>
<tr>
<td>17 (Carcass)</td>
<td>9.0 days</td>
<td>6.9 days</td>
<td>0</td>
</tr>
</tbody>
</table>

*See text for method of calculation of kinetic parameters.

*Transit time: time an average vitamin A molecule spends in a given compartment in a single transit.

*Residence time: total time an average vitamin A molecule spends in a given compartment before irreversible loss.

*Recycling number: total number of times an average vitamin A molecule recycles through a given compartment before irreversible loss.
LIVER PLASMA EXTRAHEPATIC TISSUES

Fig. 4. Model-predicted compartment masses [µg; M(i) shown within compartments] and transfer rates [µg/day; R(i,j) = M(j) * L(i,j)] from Fig. 3. U(3) = system input rate, µg/day.

The methods of model-based multicompartmental tracer kinetic analysis have not been previously used to study the dynamics of vitamin A turnover in either experimental animals or humans. We applied these techniques to the study of retinol kinetics in the moderately vitamin A-sufficient rat, and have developed a multicompartmental model that is compatible with present turnover data and with many prevailing concepts in the field. In this study, invasive tissue sampling provided data on tissue tracer responses, and facilitated development of a detailed whole body mechanistic model. The kinetic model presented describes vitamin A metabolism in the rat, but since it is believed that many aspects of the vitamin's metabolism are qualitatively similar in humans and rats (e.g., absorption of dietary vitamin A, transport to and uptake by the liver, and plasma transport), some of the implications of this model may also apply in humans. This possibility will require future investigation.

Two methodological aspects of this study deserve emphasis: the dose administered to recipient rats was small in mass, thus causing only a minor perturbation of the tracee system, and primarily physiological in form; and the studies were carried out long enough to insure equilibration of the tracer with slowly turning-over tracee compartments. In addition, interpretation of our results emphasizes several previously unrecognized aspects of the dynamics of vitamin A metabolism and suggests ideas for continued development and testing of the model.

The mathematical modeling approach used in the present studies is distinguished by several important features (3, 23) including its utilization of information from previous studies to both develop an initial model and constrain certain parameters; its capacity to simultaneously consider data from tissue subsystems at various times (in addition to that describing the plasma); and its ability to solve systems in which the tracee is not in a steady state. In the present model, we assumed that, if a slight negative vitamin A balance did exist, the rate constants remained time invariant. Although this assumption will require testing in future experiments, time-invariant rate constants were consistent with the data.

In the initial stages of modeling, we considered the possibility that a fraction of the irreversible loss of vitamin A might be due to nonfunctional disposal from the plasma...
ROH compartment. That is, we hypothesized that there might be excretion of vitamin A without prior conversion to an irreversible polar metabolite (e.g., following kidney filtration of holoRBP and incomplete reabsorption). We thus tested a model that had a direct loss of retinol from plasma compartment 11 (e.g., through kidney filtration), in addition to the irreversible loss of vitamin A from tissues. The extra parameter did not reduce the weighted residual sum of squares, and thus the model with added filtration of holoRBP and incomplete reabsorption) was very different for the two models. Thus, in order to further investigate the possibility of nonfunctional disposal, tissues would need to be sampled at various early times.

To test the effect of our assumption that irreversible loss of tracer was in proportion to the size of the rapidly turning-over vitamin A compartments in the liver, kidney, and carcass [i.e., that \( L(0,5) = L(0,13) = L(0,16) \)], we compared our model to the three extreme cases in which all output was from either the liver, or the kidneys, or the carcass. Using the Akaike Information Criterion, the models in which all output was from either the liver or the kidneys fit the data as well as the final model presented. The model with all output from the carcass did not provide as good a fit. However, the system disposal rate, total traced mass, model-predicted mass of vitamin A in various tissues, and the extent of vitamin A recycling were similar in all cases. For example, the system disposal rate varied by less than 4% among the models, with the largest value occurring if all irreversible loss was from the liver. Similarly, we checked our assumption that the rate constants between the slower and faster kidney compartments, and for recycling to the plasma, were equal to those for the carcass [i.e., that \( L(14,13) = L(17,16) \), \( L(13,14) = L(16,17) \) and \( L(11,13) = L(11,16) \)]. Freeing these parameters and thus increasing the number of adjustable parameters by three did not reduce the residual sum of squares or markedly influence any of the kinetic parameters: the system disposal rate was 6.98 μg/day (vs 6.92 in the fixed-parameter model); the total traced mass was 144 pg (vs 143) and the distribution of vitamin A in liver versus extrahepatic tissues was 71/73 μg (vs 70/73). These results are not surprising since the kidney vitamin A mass is small in comparison to the whole system, and since the kidneys made only a minor contribution to the overall system kinetics.

The model predicted a utilization rate (irreversible disposal rate) of 6.9 μg of REq/day. This value is slightly higher but in the same range as that determined using a variety of kinetic methods in vitamin A-sufficient rats (3-5 μg/day; 24-27). Lewis, Green, and Underwood (28) calculated a disposal rate of 7 μg/day in rats with lower liver stores (11 μg). Technically, our calculated disposal rate would be a maximum value if there were any chemical transformations in vivo that led to loss of tracer without irreversible loss of tracee (e.g., isomerization of all-trans retinol to 11-cis-retinol; 29, 30). Since this process seems to be important only in the neural retina, isomerization presumably would make at most a minor contribution to whole body retinol dynamics, especially if there is not extensive recycling of ROH from the retina.

It is of interest to emphasize that the estimated disposal rate was only slightly lower than estimated dietary intake (9 μg/day). Thus, either absorption of dietary vitamin A was extremely efficient (77% if rats were in vitamin A balance), or absorption was lower and animals were in a slightly negative balance for at least part of the study. Retinol absorption in the rat has been estimated to range between 37 to 48% (31-33) and 58 to 68% (M. H. Green and J. B. Green, unpublished results).

A variety of types of studies support the idea that there are several kinetically distinct pools of vitamin A in the liver (28, 34, 35). The current data required a minimum of two pools in the liver; one (compartment 3) was smaller in mass (2% of total liver vitamin A) and more rapidly turning-over [transit time = 0.024 d (0.6 hr)]; the other larger pool (compartment 5) had a transit time of 3 days. Although we did not investigate the cell types involved in hepatic vitamin A storage, or the distribution of ROH versus RE in liver compartments 3 and 5, our data are consistent with the suggestion that compartment 5 is equivalent to retinyl ester storage in both hepatocytes and stellate cells (34-37). We hypothesize that vitamin A in liver compartment 3 represents intra-hepatocyte retinol and rapidly turning-over retinyl esters, and any recycled plasma holoRBP bound to proposed cell surface receptors. In view of the apparent rapid transfer between plasma and liver ROH, and liver ROH and RE, and in view of the high uncertainty for liver-related parameters in the current model \([L(3,11), L(11,3), L(5,3)\) and \(L(3,5)\)], it will be important in future experiments to sample the liver several times in the first few hours after dose administration.

A minimum of two pools was required to fit turnover data for extrahepatic tissues (kidney as well as remaining carcass). The small rapidly turning-over retinol pools in the kidney (compartment 13) and carcass (compartment 16) may correspond physiologically to intracellular ROH or RE, and to any nonperfused interstitial holoRBP or holoRBP interacting with cell surface receptors. Of particular interest was the model's prediction that about half of whole body vitamin A mass was present in extrahepatic tissues. This observation is consistent with an overestimate of carcass retinol mass calculated from terminal plasma specific activity and radioactivity present in car-
cass (dpm in carcass + dpm/µg ROH in plasma = 103 µg). Assuming this prediction is substantiated in subsequent studies using improved analytical methods (i.e., with appropriate internal standards and measurement of tissue levels of retinyl esters vs retinol), it will be of interest to determine the potential role of extrahepatic vitamin A stores in whole body retinol dynamics. If the size of extrahepatic pools of vitamin A is dependent on vitamin A intake, total body disposal rates calculated from liver retinol depletion rates (25–27) would underestimate the actual value.

A number of investigators (38–42) have shown that the kidney plays an important role in RBP catabolism. Our model predicted that an average retinol molecule was not taken up by the kidney, but the data are consistent with the possibility that there is substantial filtration and reabsorption of retinol from kidney tubules (40, 43). Assuming 3.5% of plasma holoRBP is not bound to TTR (42, 44), one can calculate that 16 ng ROH/ml plasma is potentially subject to kidney filtration. If fractional clearance of free holoRBP by the kidney is approximately 0.5 (45) and glomerular filtration rate is 0.96 ml/min per g kidney (46), about 34 µg of ROH is filtered/day. Although numerically this is equal to 39% of total plasma turnover, the ROH acts kinetically as if it never left the plasma (that is, the ROH must have been rapidly reabsorbed from a small proximal tubule compartment). In fact, when we simulated such a compartment in the model with a mass equivalent to 15-min worth of estimated ROH filtration (= 0.35 µg), it had no effect on the plasma 3H-labeled ROH response. In addition, this compartment would have contained less than 10% of the total kidney tracer at all times sampled. Since we observed only small amounts of radioactivity in plasma RE (compartment 21) and an average transfer rate of only 0.7 µg of REq/day, our data support the speculation that, after glomerular filtration and proximal tubule reabsorption, the RBP molecule is degraded and retinol is recycled to plasma on a different RBP molecule, not as RE on plasma lipoproteins. From the estimate that 34 µg of ROH is filtered/day, one can calculate a filtration rate of 3.5 mg of RBP/day. If all this RBP was degraded, the minimum kidney RBP degradation rate would be 0.58 mg/100 g body weight per day.

The present study substantiates and quantitates the previous suggestion (26, 28, 39) that there is extensive recycling of retinol among plasma, liver, and extrahepatic tissues. The model predicted that 55% of the input of holoRBP into plasma was from the liver and 45% from other tissues; and that, of the liver secretion of holoRBP, more than half was from recycled plasma ROH (vs from liver stores or diet). Based on the model, we estimated hepatic extraction of holoRBP [L(3,11)] and the extraction fraction as follows: if 92.8% of the plasma volume flows through the liver/min (47), L(3,11) would equal 0.93/min if 100% of holoRBP was extracted on each pass through the liver. Since the calculated value for L(3,11) was 0.0042/min, the extraction fraction is 0.4%. However, even with a low extraction fraction, there is a tremendous recycling of holoRBP to the liver each day, and this provides the primary source of ROH for hepatic holoRBP input into plasma.

Based on our data, we cannot determine whether liver secretion of retinol as holoRBP required synthesis of new RBP, or whether RBP, like ROH, recycled through compartment 3. If secretion of holo RBP did require de novo synthesis of RBP, one can estimate an RBP synthetic rate of 0.81 mg of RBP/100 g body weight per day, based on the model-predicted value for the hepatic ROH secretion rate [R(11,3) = 48 µg of ROH/day]. (This assumes that retinol is not limiting and that all synthesized RBP is secreted.) The estimated RBP synthetic rate is similar to the figure calculated above for kidney RBP degradation rate (0.58 mg/100 g per day). For comparison, Peterson et al. (48) calculated an RBP synthetic rate of 1.49 mg/100 g per day in normal rats. Taken together, our estimates of RBP synthesis and degradation rates indicate that little of the RBP associated with ROH recycling to the liver is degraded in the liver; rather, it too is recycled to plasma. Based on these recycling estimates and the observation (10) that the ROH saturation of RBP is 60–70% in the rat, it is likely that there are adequate levels of apoRBP in plasma to complex with reabsorbed kidney ROH.

The extent of retinol recycling in rats with marginal hepatic reserves is dramatically emphasized by comparing the plasma total transfer rate (87 µg of ROH/day) and the liver secretion rate (48 µg of ROH/day) to the system disposal rate (6.9 µg/day). We suggest that this is a "high response" system, since plasma ROH input rate is 13 times the disposal rate. Thus, if extrahepatic tissue demands for ROH increased, liver recycling could decrease without an appreciable change in plasma ROH concentration or transfer rate. Similarly, since new dietary input [U(3)] represents only 9% of hepatic ROH secretion when intake is marginally adequate (as in the present studies), acute negative vitamin A balance may not affect liver secretion of holoRBP. With lower intakes, a larger fraction of liver ROH secretion would be contributed by liver retinyl ester stores, and plasma ROH concentration and transfer rates would remain constant. If input was restricted chronically, liver vitamin A storage (compartment 5) would reach a critical level of depletion and the transfer rate from this compartment [R(3,5) in Fig. 4] would be reduced; then, total hepatic ROH secretion [R(11,3)] would be reduced. This would lead to a lowered plasma ROH concentration. If decreased plasma vitamin A levels
led to reduced tissue holoRBP uptake and utilization, possibly due to changing apoRBP/holoRBP and free holoRBP/total RBP ratios (30, 44), the system disposal rate would also decrease. In addition, decreased tissue uptake of holoRBP would potentially increase the number of recyclings, increasing availability of vitamin A to critically dependent tissues. Plasma vitamin A levels and the system disposal rate would thus drop in an attempt to return the system to a dynamic steady state (input = output).

In conclusion, we hypothesize that plasma vitamin A concentrations may be maintained in a homeokinetic state by a control system which includes both hepatic and extrahepatic recycling of holoRBP. Integrating our data with the work of others, we suggest that vitamin A turnover can be described as a high response system in which changes in the dynamics of recycling of holoRBP can permit rapid adjustment in vitamin A distribution in response to changes in nutritional, metabolic, and/or physiological state.

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