Retinol metabolism in rats with low vitamin A status: a compartmental model[1]

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Abstract A compartmental model was developed to describe the metabolism of vitamin A in rats with low vitamin A status maintained by a low dietary intake of vitamin A (~2 μg retinol equivalents/day). After the IV bolus injection of [^{3}H]retinol in its physiological transport complex, tracer and tracee data were obtained from plasma, organs (liver, kidneys, small intestine, eyes, adrenals, testes, lungs, carcass), and tracer data were obtained from urine and feces. The dietary protocol developed for this study resulted in animals having plasma vitamin A levels less than 10 μg retinol/dl and total liver vitamin A levels of approximately 1 μg retinol equivalent. Four compartments were used to model the plasma: one to describe retinol, one to describe the nonphysiological portion of the dose, and two to simulate polar metabolites derived from retinol. The liver required two compartments and a delay, the carcass (small intestine, eyes, adrenals, testes, and lungs, plus remaining carcass) required three compartments, and the kidneys required two. The model predicted a vitamin A utilization rate of 1.65 μg retinol equivalents/day with the urine and feces accounting for most of the output. The plasma retinol turnover rate was approximately 20 μg retinol equivalents/day; this was 12 times greater than the utilization rate. This indicated that, of the large amount of retinol moving through the plasma each day, less than 10% of this was actually being irreversibly utilized. Similarly, as compared to the whole-body utilization rate, there was a relatively high turnover rate of retinol in the kidneys, carcass, and liver (9.0, 8.2, and 5.8 μg retinol equivalents/day, respectively), coupled with a high degree of recycling of vitamin A through these tissues. Of the total vitamin A that entered the liver from all sources including the diet, approximately 86% was mobilized into the plasma. Similarly, of the vitamin A that entered the carcass, approximately 76% was returned to the plasma. All of the retinol that entered the kidneys was modeled as recycling to the plasma. The present studies provide quantitative and descriptive evidence of an efficient metabolism of vitamin A from absorption through turnover and utilization in rats with very low vitamin A status. Furthermore, although their body stores of vitamin A were extremely low, these rats maintained a high level of recycling of vitamin A throughout the body.—**Lewis, K. C., M. H. Green, J. B. Green, and L. A. Zech.** Retinol metabolism in rats with low vitamin A status: a compartmental model. *J. Lipid Res.* 1990. **31:** 1535–1548.

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Despite major advances in the study of both regulatory and molecular mechanisms involved in the metabolism of vitamin A, many fundamental questions remain to be resolved regarding the role of vitamin A in the general physiological functions of growth and differentiation, reproduction, and vision. Specifically, it is important to elucidate the underlying homeostatic mechanisms that function to regulate hepatic release, plasma concentration, and recycling between the plasma and various tissues, as well as the tissue uptake and utilization of vitamin A. An improved understanding of these mechanisms will make it possible to better estimate how certain physiological, pharmacological, or nutritionally related perturbations affect dietary requirements for the vitamin.

One approach to delineating the dynamics of vitamin A metabolism is to study the vitamin's whole-body kinetics at different levels of vitamin A status. A study by Lewis, Green, and Underwood (1) compared the kinetics of radiolabeled vitamin A for 2 d in vitamin A-sufficient and vitamin A-deficient rats using a three-pool kinetic model. Besides obtaining quantitative information on kinetic parameters such as turnover rates and utilization rates of the vitamin, the study provided evidence of both extensive recycling of vitamin A throughout the system as well as hepatic uptake and reutilization of retinol associated with the retinol-binding protein (RBP)/transthyretin (TTR) plasma transport complex. Additionally, the data indicated that longer experiments were required to study

Abbreviations: RBP, retinol-binding protein; TTR, transthyretin; RE, 1 μg retinol equivalent; SEM, standard error of the mean; FSD, fractional standard deviation (standard deviation/mean); HPLC, high performance liquid chromatography.

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some aspects of vitamin A kinetics. Expanding on the initial research, a more recent study of rats with more sufficient vitamin A stores was carried out for a longer period, and model-based compartmental analysis techniques were used to analyze plasma and organ data (2). The work presented herein is an extension of both of these earlier studies. A compartmental model was constructed to describe the metabolism of vitamin A in rats with very low body stores of vitamin A.

METHODS

Chemicals and isotopes

All chemicals and solvents were reagent grade or high-performance liquid chromatography (HPLC) grade. Retinol, retinyl acetate, and retinyl palmitate to be used as standards or in diet preparations were used as purchased (Sigma Chemical Co., St. Louis, MO). Radiolabeled vitamin A acetate was used as received (either 11,12(n)-[^{3}H]retinyl acetate, sp act 160 μCi/μg, Amersham Corp., Arlington Heights, IL; or all-*trans*-11-[^{3}H]retinyl acetate, sp act 6.0 μCi/μg, prepared by SRI International, Menlo Park, CA for the National Cancer Institute). [^{3}H]Glucose (sp act 32 μCi/μg, Amersham) was diluted with water (1:300 v/v) prior to use. All procedures involving vitamin A were carried out under yellow light.

Animals and diets

Weanling male Sprague-Dawley rats (Hilltop Lab Animals, Scottdale, PA) weighing 50–60 g were housed individually in wire-bottomed stainless steel cages in an isolated room with automatically controlled temperature (22–26°C), relative humidity (60%), and 12-h light/dark cycles (0700–1900/1900–0700 h). Food and water were provided ad libitum.

Rats were designated as either recipients or donors. Recipients were fed a vitamin A-free diet (3) for approximately 7 weeks, resulting in individual plasma retinol levels between 2 and 7 μg/dl and total liver stores presumably in the range of 5 μg retinol equivalents (RE) or less. Recipients were then switched to a diet formulated to contain 0.1 RE (as retinyl palmitate) per gram of diet providing, on average, 2 RE per d.[3] This maintenance diet was designed for the present study to maintain plasma retinol levels at approximately 10 μg/dl and allow minimal, if any, hepatic storage of the vitamin. Depending on whether an experimental group was studied on a short-term or long-term basis, recipients were fed this maintenance diet for 27–100 d.

[3]Approximately 1.2 RE/g diet or 24 RE/d is the intake recommended for rats (4).

Donors were fed a vitamin A-free diet until their plasma retinol levels were less than 8 μg/dl, at which time we expected their hepatic stores of the vitamin to be 5 RE or less. At this point donors can be used for preparation of the dose to be administered to the recipients (described below) or they can be fed the recipient maintenance diet until needed. For the present studies, donor rats were fed the maintenance diet for 4–6 weeks until they were used for dose preparation.

Preparation of retinol-labeled plasma in donor rats

The preparation of retinol-labeled plasma in donor rats has been described earlier (1–3). A similar approach was used for the present study. Briefly, [^{3}H]retinol-labeled plasma was made by administering tritiated retinyl acetate in a Tween 40 carrier solution to donor rats. The plasma collected 90–100 min after injection of [^{3}H]retinyl acetate was ultracentrifuged to remove any unmetabolized Tween micelles. We have previously shown (2, 3) that, using this procedure, more than 90% of the [^{3}H]retinol in plasma is associated with the RBP/TTR complex. The [^{3}H]retinol/RBP/TTR-labeled plasma was stored under nitrogen atmosphere at 4°C and used for injection into recipients within 3 days of collection.

In vivo turnover studies

For the vitamin A turnover studies, nonfasting recipient rats were anesthetized with methoxyflurane (Pitman-Moore, Washington Crossing, NJ) between 0841 and 1349 h. A baseline (t_0) blood sample (~0.1 ml) was collected from a caudal vein into tubes containing Na_2EDTA (final concentration ~4 μM). Weighed aliquots of [^{3}H]retinol/RBP/TTR-labeled plasma (0.5–1.0 g) were then injected into a jugular vein and anesthesia was removed. After injection, serial blood samples (0.15–0.20 ml) were collected from a caudal vein until the rats were killed. Aliquots of plasma were stored at −16°C under nitrogen atmosphere until analysis.

In vivo turnover studies were done in groups of rats killed at 12 min (n = 3), 2 h (n = 3), 6 h (n = 3), 10 h (n = 3), 3 d (n = 3), 4 d (n = 3), 5 d (n = 3), 23 d (n = 5), 28 d (n = 3), and 35 d (n = 5) after administration of retinol-labeled plasma. Estimates of optimal times for blood sampling and organ collection were determined using sensitivity analysis (5–7) and data from previous experiments. The blood sampling schedule for rats in the 23, 28, and 35 d groups was based on a geometric progression from 9 min to 35 d (40–41 samples); for other groups, a small number of blood samples (n = 1–5) was collected before killing.

At the time of killing, rats were anesthetized with methoxyflurane, and whole body perfusion was done by gravity feed (1.9 m) of ~200 ml of 0.9% saline solution containing 0.2% D-isoascorbic acid (Sigma); perfusate was circulated from the left cardiac ventricle to the right

auricle. After perfusion, the following organs were removed for analysis: liver, kidneys (decapsulated), small intestine (rinsed of contents), eyes (enucleated), adrenals, testes, and lungs. Tissues were blotted, weighed, frozen, lyophilized (except for eyes and adrenals), and stored (−16°C) in sealed plastic bags purged with nitrogen until analysis. Remaining carcasses (minus contents of stomach, large intestine, and urinary bladder) were also stored (−16°C) in sealed plastic bags purged with nitrogen until processed for analysis.

Metabolic studies

For several groups of rats (6 h, 3 d, 5 d, and 23 d groups), a separate set of experiments was done. This consisted of a series of metabolic studies that involved the collection of urine and feces samples during the course of the turnover studies carried out in these same groups. Collection times for urine and feces were based on the results of pilot studies. Recipient animals used for the metabolic studies were treated similarly to those used in the other turnover studies in regard to dietary treatment, injection procedures, and blood and organ collections, except that the metabolic study groups were fed the maintenance diet for a longer period (86–100 d as compared to 27–45 d). After removal of a blood sample at approximately 5 min after injection of labeled donor plasma, rats from the 6 h, 3 d, and 5 d groups were placed in metabolic cages (Nalge Co., Rochester, NY) to which they had previously been adapted. Urine collections were done at 1.5, 3, 4.5, 6 and 12 h and then at 12-h intervals for up to 5 d; fecal collections were done at 6 and 12 h and then at 12-h intervals for up to 5 d. Rats in the 23-d group were placed in metabolic cages at 10 d post-injection and urine and fecal collections were done at 2-d intervals between 12 and 22 d, with a final collection between 22 and 23 d. At each collection time the rats were moved to a clean, tracer-free metabolic cage. After removal of the urine and feces collection tubes, cages were rinsed thoroughly with approximately 100 ml of a mixture of absolute ethanol and distilled water (1:1 v/v). Rinses were combined with urine and aliquots were stored at −16°C until analysis. For feces, adhering hair and food was removed and samples were weighed, frozen in plastic bags, lyophilized, and stored at −16°C until analysis.

Plasma and organ processing and extractions

An internal standard [retinyl acetate in absolute ethanol containing 5 μg/ml butylated hydroxytoluene (BHT); Sigma] was added to plasma samples to be analyzed for vitamin A mass. Plasma samples (0.05–0.1 ml) and aliquots of the injected doses were extracted (8, 9) from 2 ml of 50% aqueous ethanol into 4.5 ml of hexane containing BHT (5 μg/ml). After vortexing and centrifugation, appropriate aliquots were removed for analysis by HPLC and/or liquid scintillation spectrometry.

Frozen carcasses were cut into approximately 125 cm^3 pieces and homogenized (Model CB-6, Waring Products Division, New Hartford, CT) using 500 ml of distilled water containing 0.1% isoascorbate as an antioxidant. Aliquots of carcass homogenate (2–3 g) and of freeze-dried liver, kidneys, small intestines, testes, and lungs (0.25–0.35 g) were extracted using a modification of the hexane–isopropanol method of Hara and Radin (10, 11) developed for the present studies. This method was tested in an earlier study and used for the analysis of liver samples (3). Retinyl acetate internal standard was added to aliquots of organ sample, 18 ml of hexane–isopropanol 3:2(v/v) containing BHT (5 μg/ml) was added, and samples were vortexed for 2 min. To this mixture was added 9 ml of sodium sulfate wash solution (66.7 g/l of water) after which the mixture was vortexed for 1 min followed by centrifugation at low speed for 7 min. After centrifugation, the upper hexane layer was removed and set aside, and the aqueous phase was extracted 2 more times with 4 ml each of hexane–isopropanol 7:2(v/v) containing BHT (5 μg/ml). For each of these latter two extractions, the upper hexane layer was removed and pooled with the first. The pooled hexane extracts were brought up to constant volume (25 ml) and appropriate aliquots were removed for analysis of vitamin A mass and/or radioactivity. Whole eyes and adrenal glands were similarly extracted using a scaled-down version of the above method.

Urine and feces analysis

Weighed aliquots (~1 g) of urine were put into glass counting vials and solubilized in scintillation fluid in preparation for analysis of radioactivity.

Fecal samples for rats in the 23 d group were homogenized in a volume of water 7 times their dry weight. Aliquots of these homogenates or lyophilized fecal samples collected from the other groups were combusted in a biological materials oxidizer (Model OX-400, Harvey Instrument Co., Hillsdale, NJ) and the resulting tritiated water was trapped in scintillation fluid. Recovery of radioactivity was determined for each set of sample replicates by adding a known amount of [^{3}H]glucose to triplicate sample aliquots prior to combustion. Triplicate aliquots were also combusted directly.

Chromatography

Samples were prepared for HPLC by first evaporating solvent from aliquots of lipid extracts in a 37°C water bath using a gentle stream of nitrogen. After drying, samples were immediately resolubilized with absolute ethanol (plasma, liver, kidneys, eyes, adrenals, testes, lungs) or isopropanol (small intestine, carcass) prior to HPLC injection. Retinoid compounds of interest were separated by reverse-phase HPLC (Model 6000A, Model U6K Universal Injector; Waters Associates, Milford, MA) using

methanol–water as mobile phase and a Resolve C18 column (4.6 mm ID × 15 cm; Waters) equipped with a C18 guard column (Waters) for the stationary phase. For plasma samples, retinol and retinyl acetate were eluted using methanol–water 95:5(v/v) at a flow rate of 1 ml/min. For tissues, retinol and retinyl acetate were either eluted using the same mobile phase (eyes and adrenal glands) or methanol–water 90:10(v/v); then the mobile phase was switched to 100% methanol and, simultaneously, the flow rate was increased to 2 ml/min to elute longer-chain retinyl esters. Detection of retinoids was accomplished by UV absorbance at 340 nm (Models 440 and 441; Waters) and peak areas were quantitated by digital integration (Model 3390A, Hewlett-Packard, Avondale, PA; Model C-R3A, Shimadzu Corporation, Kyoto, Japan) using the mass to area ratios of the appropriate chemical standards. These standards were quantitated at 325 nm (Acta CIII, Beckman Instruments, Fullerton, CA) using extinction coefficients ($E^{1\%}_{1cm}$) of 1835 and 1560 for retinol and retinyl acetate (12), respectively, and 940 for retinyl palmitate (13). Except for the presence of retinyl palmitate and retinyl stearate in eyes and adrenals, retinyl esters were not detected in the other tissues analyzed.

Analysis of radioactivity

For determination of radioactivity in plasma and tissues, solvent was evaporated from lipid extracts of the samples which were then resolubilized in toluene containing 0.4% 2,5-diphenyloxazole. Urine samples were counted in a premixed scintillation cocktail (Liquiscint; National Diagnostics, Manville, NJ) as were feces samples (Ready-Solv; Beckman Instruments, Irvine, CA). Tritium in plasma, tissues, urine, and feces samples was analyzed by liquid scintillation spectrometry.

Samples were counted twice (Models 3801 and 8100, Beckman Instruments; Model 3385, Packard Instruments, LaGrange, IL; Model SL-30, IN/US Service Corp., Fairfield, NJ) to a 2-sigma error of 2% or a maximum counting time of 100 min. Sample cpm were converted to dpm. Quenching was corrected for using an external standard method. Recipient and donor plasma samples were counted within 8–10 weeks of collection. Other samples counted beyond this point were corrected for physical decay of ^3H label.

Kinetic analysis and modeling

To calculate tracer concentration in plasma at the time of dose administration (t_0) for each recipient, the radioactivity injected (dpm in dose) was divided by the estimated plasma volume [where plasma volume (ml) = t_0 body weight (g) * 0.04 ml of plasma/g of body weight (14)]. The dose was sequentially adjusted for the amount of radioactivity removed in prior blood samples (adjusted dose). To calculate the fraction of dose in plasma, the measured dpm/ml at each time was divided by the adjusted dose dpm/ml. For kinetic analysis, the geometric mean fraction of dose in plasma at each time was calculated (15) for rats in the 28 and 35 d groups. Plasma data for rats killed at other times were examined to verify that they showed similar patterns, but they were not used in model identification.

The geometric mean fraction of dose in tissues, urine, and feces was calculated for each collection time, as was the cumulative fraction of dose recovered in urine and feces between 0 and 5 d, and between 12 and 23 d. The standard error of the data mean (SEM) at each time (or 5% of the geometric mean if SEM was smaller than 5% of the mean) was used as the weighting factor for nonlinear regression analysis during model development, except a weight of 10% of the geometric mean was used for the cumulative urine and feces data. The average vitamin A mass in tissues was calculated from the 28 and 35 d group means and used as an estimate of tissue vitamin A content during the turnover study. The average plasma retinol pool size (μg) used in model development was based on determinations (n = 10) of plasma retinol concentrations (μg/dl) and estimates of plasma volumes (ml) monitored throughout the turnover study for rats in the 28 and 35 d groups.

Kinetic analysis and subsequent mathematical modeling of plasma and tissue tracer and tracee data and urine and feces tracer data were done using the Simulation, Analysis and Modeling (SAAM 29) (5) and Conversational SAAM (CONSAM) (6) computer programs on a VAX 8350 (Digital Equipment Corp., Marlboro, MA). For model development we combined the data for the small intestine, eyes, adrenals, testes, and lungs with the carcass data to form "lumped carcass" tracer and tracee data sets. Similarly, we minimized the number of adjustable parameters by first estimating and then fixing certain parameters, and establishing dependency relationships between others. Fixed or dependent parameters included in the model were compatible with current knowledge of vitamin A metabolism. For modeling, we assumed that the tracer data could be simulated as the solution to a compartmental model with time-invariant fractional rate constants. To develop the model hypothesized in the present report, the parameter values and model structure of a starting model were adjusted using SAAM/CONSAM to obtain a consistent, best fit of model-predicted and observed data. A 4-component multiexponential equation describing the 28 and 35 d group plasma radioactivity versus time data was used as a forcing function (5, 6, 16) to provide input into the model subsystems (liver, kidneys, and carcass) and these subsystems were initially modeled separately. During the later stages of model development, the forcing function was removed and the entire data set was modeled simultaneously. Final parameter values and estimates of their fractional standard deviation (FSD) were obtained using an iterative, nonlinear least squares procedure (5, 6).

Nomenclature and model-based kinetic parameters

The nomenclature and method of calculation of kinetic parameters is based on work described by several groups (17–22). The parameters that were calculated based on the model included the fractional rate constants [L(I, J); d^{-1}], or the fraction of the mass in compartment J [M(J); RE] that is transferred to compartment I per unit time. The mean transit time [$\bar{t}$ (I); h] is the average interval of time during which the molecules of the compound of interest that reach compartment I stay in compartment I during one passage through this compartment and is obtained by:

$$\bar{t}\,(I) = \frac{1}{\sum_{\substack{J=0 \\ J \neq I}}^{N} L(J,I)} = [1/L(I,I)] * 24.$$

The mean residence time, [$\bar{T}$ (I,J); h], is defined as the total amount of time, on average, that a molecule originating in (or entering) compartment J is expected to spend in compartment I before irreversible loss and is calculated by:

$$\bar{T}\,(I,J) = \{1/FCR(I,J)\} * 24$$

where FCR(I,J), or fractional catabolic rate, is the fraction of molecules in compartment I that leave irreversibly per day after introduction into the system via compartment J [U(J); where U represents the steady state input into the system]. Compartment residence times were obtained from the inverse matrix of the model L(I,J) as calculated by CONSAM. For determination of residence times in this manner, it was assumed that tracee molecules entered the system via liver compartment 3. Thus, the residence time for a compartment is distinguished from the transit time in that it represents a mean time for all material in the system and is, in part, dependent on where this material enters the system. In contrast, the transit time is concerned only with how long material that is in the compartment (or that which reaches the compartment) will remain there before exiting the compartment.

The number of cycles through compartment I, C(I), or the number of times a tracee molecule cycles through a compartment of interest before it is irreversibly lost from that compartment, is obtained by:

$$C(I) = [\bar{T}\,(I,J)/\bar{t}(I)].$$

The difference between cycling and recycling as used in the present report is that cycling includes all passes through a compartment whereas recycling does not include the initial pass through the compartment (i.e., number of cycles − 1= number of recyclings).

The turnover (or transfer) rate [R(I,J); RE/d] is the amount of steady state tracee material transferred to compartment I from compartment J per unit time and is calculated as L(I,J) * M(J). The turnover rate for a compartment, R(I,I), is the amount of steady state tracee material moving through compartment I per unit time and is calculated as L(I,I) * M(I).

The tissue transit time, [$\bar{t}_{(tissue)}$], is defined as the average amount of time a molecule of vitamin A that enters the tissue spends in all compartments that comprise the tissue before leaving reversibly or irreversibly. It is calculated by:

$$\bar{t}_{(tissue)} = M_{(tissue)}/R(I,I)$$

where $M_{(tissue)}$ is the steady state mass of vitamin A in the tissue and R(I,I) is the sum of the transfer rates out of the tissue. This calculation is valid only for tissues with a single site of input.

RESULTS

Animal outcome

Rats (n = 34) weighed 430 ± 29 g (mean ± SD) at the beginning of the turnover studies. Those in the 28 and 35 d groups weighed 435 ± 20 g at the beginning of the study and at the time of killing their mean body weight averaged 451 ± 18 g (n = 8). Despite their low vitamin A intake (0 RE/d for 45–50 d, then ~2 RE/d), rats in this study gained weight and appeared to be in good health. Comparison of growth curves for animals used in the present study and a more vitamin A-sufficient group studied earlier (3) revealed no apparent differences in growth rate between the two groups.

Plasma retinol concentrations for all recipients averaged 10.6 ± 2.2 μg/dl (n = 34) at the time of dose administration and 9.9 ± 1.2 μg/dl (n = 8) at the time of killing of the 28 and 35 d groups. The vitamin A mass in the donor plasma injected caused a perturbation of approximately 24% of the estimated recipient plasma vitamin A pool. The plasma retinol levels of the 28 and 35 d groups did not vary much during the turnover study; the coefficient of variation was 7 to 18% for 10 sampling times. Most of this variation could be accounted for by the tendency of plasma retinol levels to decline slightly post-injection followed by a return to pre-dose levels.

The average mass of vitamin A as measured by HPLC in the five rats killed at 35 d was 0.50 ± 0.10 RE for the liver, 0.60 ± 0.10 RE for the kidneys, and 15.7 ± 8.7 RE for the carcass. The average vitamin A values for these tissues in three rats killed at 12 min post-dosing were significantly higher than those measured at 35 d ($P < 0.05$; as determined by independent *t*-test); the average value

for liver was 2.18 ± 0.23 RE, kidneys 2.13 ± 0.21, and carcass 56.5 ± 9.5 RE. The differences in mass between these two groups suggested that rats were in a negative vitamin A balance during the 35-d experimental period.

Kinetic data

Group average data for plasma vitamin A radioactivity versus time post-dosing are presented in **Fig. 1**. Following an initial rapid disappearance of label which lasted for 1.5–2 h, there was a more gradual decline which persisted to approximately 16–18 d. This was followed by a period during which the slope appeared to decrease very slowly until the terminal collection point.

The plots for liver, kidneys, and carcass fraction of injected dose versus time are shown in **Fig. 2**. The recovery of radioactivity in the liver and carcass peaked in the 10 h group (5.9 ± 0.3% and 44 ± 2.2%, respectively) whereas the kidney radioactivity peaked earlier as reflected in the 2 h group (18 ± 0.9%). At 35 d only about 5% of the injected radioactivity was recovered in the plasma and tissues; thus about 95% of the dose had been lost from the body by this time.

The plots of urine and feces cumulative radioactivity versus time post-dosing in **Fig. 3** show that 24% of the injected dose was excreted in the urine and 21% of the dose appeared in the feces within the first 5 d.

As an estimate of variance for the data shown in Figs. 1–3, we calculated (SEM/geometric mean) * 100 at each time. These values ranged from 2 to 16% for the plasma, 2 to 17% for the liver, 2 to 12% for the kidneys, and 2 to 15% for the carcass. The corresponding values for the urine and feces were 5–28% and 8–55%, respectively.

Model development and proposed model

A conceptual model (23) and a general kinetic model (1) of vitamin A metabolism proposed previously were used to provide initial trial compartmental models. In the later stages of development of the present model, a more recent model describing vitamin A metabolism in rats with marginally sufficient vitamin A status (2) was referred to for comparison purposes. Three compartments were hypothesized to describe retinol metabolism in the initial stages of modeling. In this first model, retinol as part of its trimolecular transport complex was mobilized from the liver (one compartment) into the plasma (a second compartment), after which it was distributed to the remaining tissues (the third compartment). The model included recycling of retinol between the plasma and the liver as well as recycling between the plasma and remaining tissues. System input was into the liver, corresponding to hepatic

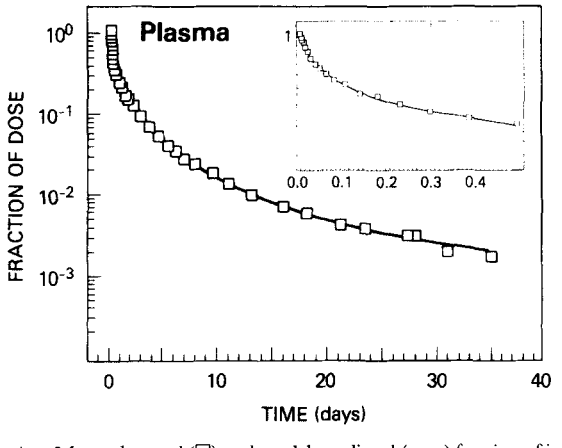

Fig. 1. Mean observed (□) and model-predicted (——) fraction of injected dose in plasma versus time for rats (n = 8) killed 28 and 35 d after administration of [^{3}H]retinol-labeled plasma. The inset shows the data from the first 12 h on an expanded time scale and one cycle on the semilog plot.

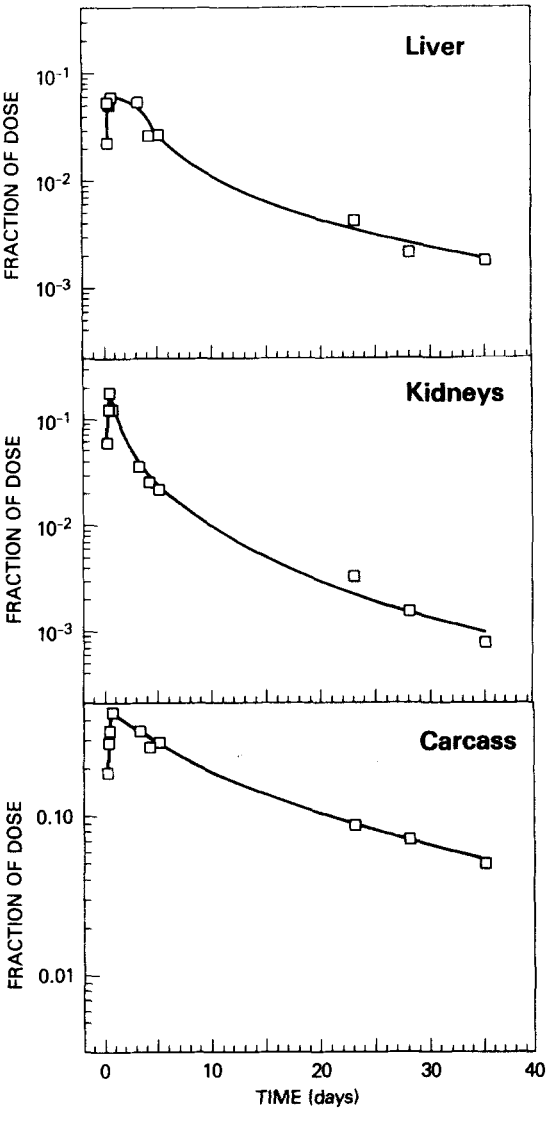

Fig. 2. Mean observed (□) and model-predicted (——) fraction of dose in liver (top panel), kidneys (middle panel), and carcass (lower panel) versus time after injection of [^{3}H]retinol-labeled plasma. "Carcass" includes small intestine, eyes, adrenals, testes, lungs, and remaining carcass tissue (see Methods). Three to five rats were killed at each time (see Methods).

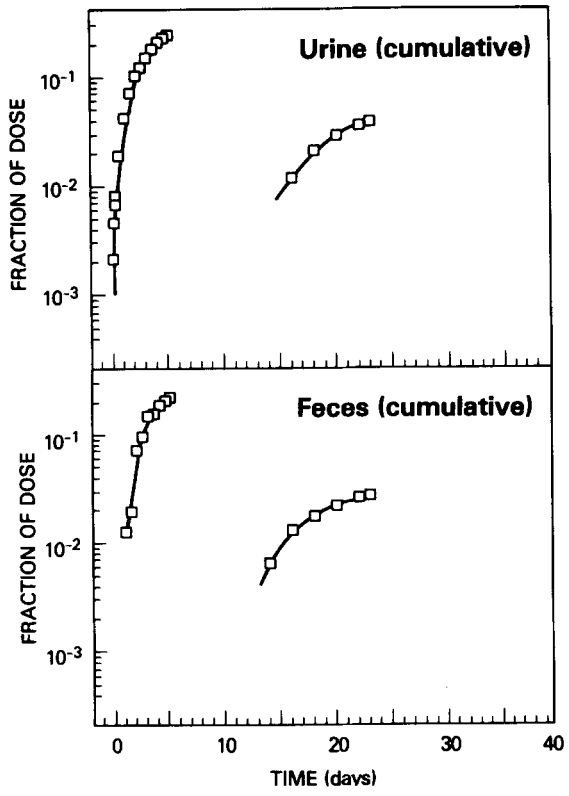

Fig. 3. Mean observed (□) and model-predicted (——) cumulative fraction of dose in urine (top panel) and feces (bottom panel) versus time after injection of [^{3}H]retinol-labeled plasma. Samples were collected from rats (n = 9) on a short-term basis between 0 and 5 days, and from five other rats between 12 and 23 days (see Methods).

clearance of chylomicron remnants containing diet-derived vitamin A; system output was hypothesized as occurring from the plasma (via kidney filtration) and from the liver (via biliary output into the feces). These outputs reflected the irreversible conversion of retinol to retinoic acid and other polar retinoids, and their excretion. As model development proceeded, it became clear that the observed tracer data were not compatible with this initial model when all the data were included. Ultimately the plasma required four compartments, the liver required two and a delay element, the kidneys required two compartments, and the carcass required three.

The model hypothesized to describe the current data on on vitamin A turnover in rats with low vitamin A status is shown in **Fig. 4**. The fractional rate constants and other model elements are presented in **Table 1**. The fractional standard deviation values shown in Table 1 indicate that, for most of the parameters, the model was compatible with the observed data. As will be described below, several components of the model were simulated within the context of the present model. However, since no direct sampling of these components was done in the present study several parameters associated with these components were not able to be clearly identified in the present model as indicated by their higher fractional standard deviations. These parameters included several associated with flow into the fecal delay [L(18, 21)] or urine delay [L(20, 21), L(20, 8)], as well as one parameter associated with the metabolite delay [L(14, 1)]. Important physiological parameters calculated from the primary parameters of the model are presented in **Table 2**. The model includes dietary input into the system via the liver, following absorption of dietary vitamin A and chylomicron clearance, and output from the system via the urine and feces. Additionally, a relatively small but consistent loss not able to be accounted for in the urine and feces is shown in the model associated with the carcass.

The plasma is represented by compartments 11, 19, 21, and 8 (Fig. 4). Compartments 11 and 19 were the sites of introduction of the dose. Compartment 11 represents plasma retinol associated with RBP and TTR. The transit time for plasma retinol was 1.7 h and the residence time was 20.8 h. The turnover rate of plasma retinol (compartment 11), 20.3 RE/d, was the highest estimated in the present model. Compartment 19 represents a portion of the dose that was nonphysiological in the sense that it did not act as native retinol-RBP. As predicted by the model, the material in compartment 19 made up 1.8% of the injected dose [1-P(11); Table 1] and was cleared from the system very rapidly. Only the nonpolar (i.e., hexane-extractable) forms of vitamin A were monitored in the present study. However, compartments 21 and 8 were included in the model to simulate the polar forms of the vitamin that were not monitored. It was hypothesized that these compartments represented metabolites of retinol being transported through the blood. It should be emphasized that compartments 21 and 8 do not represent only one metabolite each or any metabolites in particular, but rather represent a range of retinol metabolites of varying polarities. The estimated mass in these compartments, approximately 2.8% of the total plasma retinol mass, was constrained using values reported by others (24, 25).

The liver is comprised of compartments 3, 4, and 5. The tissue transit time indicates that a vitamin A molecule entering the liver would be expected to remain there an average of 4.5 h. Compartment 3 is hypothesized to represent primarily hepatocytes which contain a pool of vitamin A that turns over very rapidly [$\bar{t}(3)$ = 32 min]. From compartment 3, retinol is likely metabolized and secreted into bile, recycled to the plasma, or moved into a slower turning-over component [$\bar{t}(4)$ = 31 h], liver component 4, shown in the model as a delay element containing four cells. We hypothesized that this delay may represent the time it takes for retinol to be esterified, hydrolyzed, and further processed by hepatocytes or a population of hepatocytes. After processing in component 4, retinol can return to compartment 3, be transferred to compartment 5, or be metabolized and secreted into bile. Like compartment 4, compartment 5 turns over very slowly [$\bar{t}(5)$ = 30 h]. Trans-

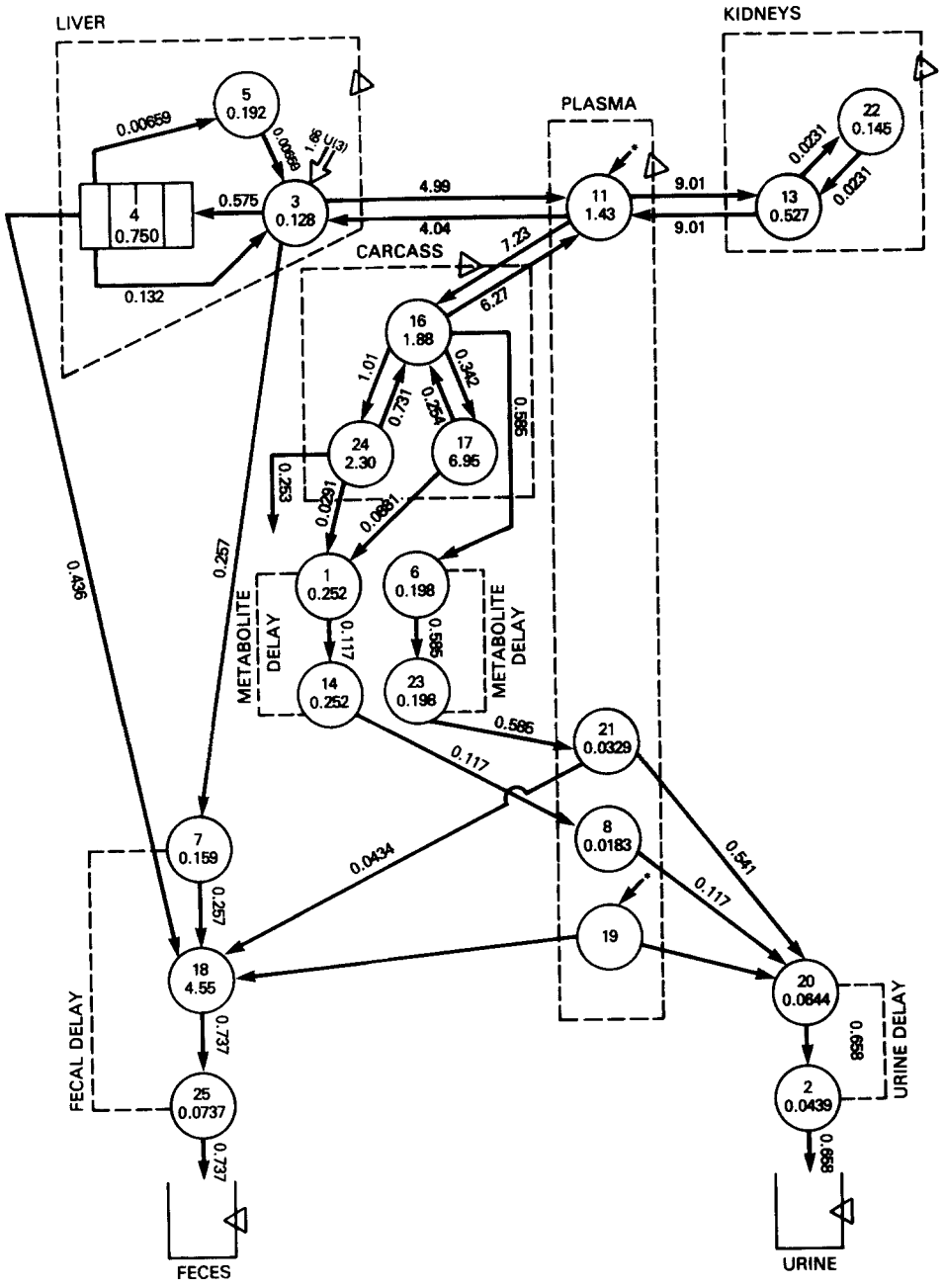

Fig. 4. Proposed model for vitamin A turnover in rats with low vitamin A status. Compartments are represented by circles, the liver delay as a rectangle with four cells, and sites of sampling by small triangles. Numbers between the compartments are turnover or transfer rates [R(I,J); RE/d] and compartment masses [M(I); RE] are shown within compartments. The asterisk denotes site of injection of donor plasma containing [^{3}H]retinol-RBP-TTR complex. U(3) denotes input of retinyl ester into the liver from the diet via chylomicron remnants.

fer to compartment 5 may represent an interchange of retinol between liver parenchymal and perisinusoidal stellate cells.

The kidneys are represented by compartments 13 and 22. Compartment 13 is hypothesized to include both filtration and subsequent reabsorption processes. It exchanges vitamin A very rapidly with plasma compartment 11. This rapid exchange is reflected in both the short transit time [$\bar{t}$ (13) = 1.4 h] and the relatively high number of cyclings (5.5 cycles) through compartment 13 prior to irreversible loss.

The carcass is represented by three compartments, 16, 17, and 24. Compartment 16 turns over very rapidly [$\bar{t}$ (16) = 5.5 h] and exchanges with plasma compartment 11 and the other two carcass compartments, 17 and 24. The latter two compartments represent slower turning-over

TABLE 1. Model parameters for proposed model of vitamin A turnover

Parameter[a]	Value	Fractional Standard Deviation[b]
Adjustable		
L(16,11)	5.05E + 00	6.51E – 02
L(11,16)	3.34E + 00	1.51E – 01
L(24,16)	5.40E – 01	1.94E – 01
L(16,24)	3.18E – 01	2.05E – 01
L(17,16)	1.82E – 01	1.94E – 01
L(16,17)	3.65E – 02	6.82E – 02
L(13,11)	6.30E + 00	5.64E – 02
L(11,13)	1.71E + 01	8.55E – 01
L(22,13)	4.38E – 02	2.87E – 01
L(13,22)	1.60E – 01	1.13E – 01
L(3,11)	2.82E + 00	6.73E – 02
L(11,3)	3.91E + 01	1.10E – 01
L(3,5)	3.32E – 02	6.13E – 01
L(7,3)	2.02E + 00	7.13E – 01
L(18,4)	3.31E + 00	5.48E – 01
L(18,7)	1.62E + 00	3.21E – 01
L(6,16)	3.12E – 01	2.86E – 01
L(23,6)	2.95E + 00	1.94E – 01
L(2,20)	1.02E + 01	8.77E – 02
L(1,17)	1.27E – 02	4.50E – 01
L(14,1)	4.65E – 01	2.06E + 00
DT(4)	1.30E + 00	6.98E – 02
L(18,21)	1.32E + 00	1.49E + 01
L(20,21)	1.65E + 01	4.63E + 00
L(20,8)	6.39E + 00	1.52E + 00
Fixed		
L(20,19)	3.00E + 01	
L(18,19)	8.00E + 00	
L(0,24)	1.10E – 01	
L(4,3)	4.50E + 00	
L(3,4)	1.00E + 00	
L(5,4)	5.00E – 02	
P(16)	8.51E – 01	
P(1)	1.74E – 01	
P(11)	9.82E – 01	
Dependent		
L(21,23) = L(23,6)	2.95E + 00	
L(1,24) = L(1,17)	1.27E – 02	
L(8,14) = L(14,1)	4.65E – 01	
L(25,18) = L(18,7)	1.62E + 00	

[a]Values shown are fractional rate constants [L(I,J); d^{-1}], delay time [DT(I); d]. Unitless parameters [P(I)] describe the model in Fig. 4; P(1) represents the estimated contamination of carcass with plasma tracer as determined by modeling; P(16) represents the estimated fractional recovery of carcass tracer determined during modeling; P(11) is the estimated fraction of the initial dose in physiological plasma compartment 11. See text for details.

[b]Fractional standard deviation: standard deviation/mean.

pools of carcass vitamin A. The tissue transit time for the carcass of 37 h is much longer than that for liver or kidneys. The model includes output from all three carcass compartments into a "metabolite" delay (compartments 1, 6, 14 and 23) from which material is transferred to plasma metabolite pools (compartments 21 and 8).

During model development, we found that the carcass data could not be fit unless more tracer was present in the carcass than could have been derived from this tissue alone. That is, for animals killed up to 10 h after dosing, the total recovery of radioactivity from plasma, organs, urine, and feces averaged 107 ± 5.6%. We hypothesized that one or more of the organs analyzed was contaminated with labeled plasma that was not completely perfused at the time of killing. The inclusion of such contamination in the carcass helped to resolve these data, but such contamination was not apparent in either the liver or kidneys. Accordingly, a factor [P(1)] describing the estimated contamination of the carcass radioactivity by plasma was included with the carcass data in the model. We also included an additional factor [P(16)] in the model with the carcass data that served as an estimate of recovery of radioactivity following carcass analysis. Thus, for the model shown in Fig. 4, it was estimated that approximately 17% of the plasma radioactivity was still present in the carcass after perfusion and approximately 15% of carcass radioactivity may have been lost as a result of processing this tissue for analysis.

Output of the [^{3}H]retinol dose via the urine and feces was also monitored in several groups included in this study. Urinary output was hypothesized to occur from both of the plasma metabolite pools. It included polar metabolites and was preceded by delay compartments 20 and 2. The model predicts an initial direct output of tracer (1.4%) into the urine from plasma compartment 19, the nonphysiological portion of the dose. Irreversible disposal from the system of urinary metabolites was hypothesized to occur via compartment 2. Fecal output was hypothesized to occur via compartment 25 following a delay represented by compartments 7, 18, and 25. The fecal delay element has input from both liver compartment 3 and component 4 as well as from the plasma metabolite pool represented by compartment 21. As was the case with the urine, there was a direct output of nonphysiological material (0.4% of the dose) into the feces from compartment 19.

To estimate the mass in the various compartments as well as the rates of retinol transfer between compartments and the disposal rate of vitamin A, the mean plasma retinol mass (1.43 μg) during the turnover study for rats in the 28 and 35 d groups was used to obtain a steady state solution (5, 6) to the proposed model. The results are shown in Fig. 4. The model-predicted utilization rate was 1.65 RE/d. The plasma retinol turnover rate (20.3 RE/d) was 12 times the utilization rate. Of the plasma retinol turnover, 44% was transferred to the kidneys {[R(13,11)/R(11,11)] * 100}, 36% to the carcass {[R(16,11)/R(11,11)] * 100}, and 20% recycled to the liver {[R(3,11)/R(11,11)] * 100}. For retinol entering the plasma, the model predicts that only 25% came from the liver and 75% from extrahepatic tissues.

The model-predicted liver vitamin A mass [M(3) + M(4) + M(5)] was 1.1 RE, less than 80% of the plasma mass [(1.1 RE /1.4RE)* 100]. Most of the mass in the liver is predicted to be in component 4. Compartment 3 had

TABLE 2. Kinetic parameters for proposed model of vitamin A turnover[a]

Compartment	Turnover Rate[b]	Transit Time[c]	Residence Time[d]	Number of Cycles[e]	Tissue Transit Time[f]
	RE/d	*h*	*h*		*h*
Plasma					
11	20.3	1.7	20.8	12.3	1.7
21	0.58	1.4	0.48	0.35	
8	0.12	3.8	0.27	0.071	
Liver					
3	5.8	0.53	1.9	3.5	4.5
4	3.3	31.3	10.9	0.35	
5	0.0066	30.1	2.9	0.096	
Kidney					
13	9.0	1.4	7.7	5.5	1.8
22	0.023	150	2.1	0.14	
Carcass					
16	8.2	5.5	27.3	5.0	36.9
17	0.34	488	101	0.21	
24	1.0	54.4	33.5	0.61	

[a]The method of calculation of kinetic parameters is found in the text.
[b]Turnover rate: the amount of vitamin A moving through a given compartment per unit time.
[c]Transit time: the average period of time vitamin A molecules that reach compartment I stay in this compartment during a single passage.
[d]Residence time: the total time, on average, a vitamin A molecule spends in compartment I after entry into the system via compartment J, prior to irreversible exit from that compartment.
[e]Number of cycles: the number of times, on average, that a vitamin A molecule cycles through a compartment before irreversible loss.
[f]Tissue transit time: the average amount of time a molecule of vitamin A that enters a given tissue spends in that tissue before it leaves reversibly or irreversibly.

a turnover rate of 5.8 RE/d and the majority of this (86%) was transferred to the plasma. The predicted vitamin A mass in the kidneys [M(13) + M(22)] was 0.67 RE. Most of this was in compartment 13, which also had the highest rate of exchange with the plasma (9 RE/d). There was a very slow transfer between kidney compartments 13 and 22. The predicted mass in the carcass was approximately 11 RE. Compartment 16 was the smallest of the three carcass compartments but had the highest rate of turnover, transferring 6.3 RE/d into the plasma. Carcass compartment 17 contained approximately 7.0 RE and was also the largest compartment in the model. Finally, the model predicted a mass of 0.9 RE for the metabolite delay compartments.

DISCUSSION

The primary objectives of these studies were to establish a dietary regimen that would maintain rats in a chronic state of low vitamin A status and to then construct a whole-body kinetic model that would describe the metabolism of vitamin A at this level of vitamin A nutriture. The kinetic model was based on a series of studies in which blood and tissue samples were collected from 10 sets of animals between 9 min and 35 d after a dose of radiolabeled retinol in its plasma transport complex. For several groups of rats, a series of metabolic studies involving urine and feces collections were done along with the blood and tissue collections. Kinetic studies in vitamin A-deficient rats have been conducted previously (1, 3, 26, 27), but the work presented here is novel in several respects. Specifically, labeled vitamin A was administered in a physiological form to a relatively large number of vitamin A-depleted rats not supplemented with retinoic acid; the kinetic study was carried out for a long period and included collection of a variety of tissues, urine, and feces; and model-based compartmental analysis was used to develop and quantitate a mechanistic model compatible with the observed data. Such an approach to the study of vitamin A metabolism has not been reported before. The model proposed provides a more detailed hypothesis about vitamin A metabolism in vitamin A-depleted rats than was previously available.

The development of an animal model with chronic low vitamin A status was an important component of this work. For our studies, the ideal low vitamin A animal was one that could be maintained for a long period of time in

a steady state (i.e., vitamin A balance) and with very little vitamin A in the liver and extrahepatic tissues. Lamb, Apiwatanaporn, and Olson (28) have suggested a technique for maintaining vitamin A-depleted rats that is based on intermittent feeding of retinoic acid, a partial functional substitute for vitamin A. However, because we wanted to investigate vitamin A kinetics under chronic conditions of low vitamin A status, we were concerned that the addition of retinoic acid to the diet would alter normal whole-body retinol kinetics. In a series of pilot studies, we confirmed that weanling rats could be depleted of nearly all of their liver vitamin A by consuming a vitamin A-free diet for 7 weeks and found that they could then be maintained for extended periods of time in apparent good health by a diet that provided ~2 RE/d. Plasma retinol levels were stable at ~10 μg/dl on this maintenance diet and there was no evidence of hepatic or extrahepatic net storage of the vitamin. However, as was pointed out earlier, these animals appeared to be in a negative balance with regard to vitamin A intake. We suggest that with a slight increase in dietary vitamin A intake to offset this negative balance, this animal model should be a useful and convenient one for future studies of vitamin A metabolism under chronic conditions of very low vitamin A status.

In order to construct the present model we postulated that the vitamin A masses in the various compartments were not changing with time in such a way that the fractional rate constants were time-variant. Based on measurement of the vitamin A content of the diet and estimates of food intake, we estimated rats were consuming ~2 RE/d. The model predicted a whole-body utilization rate of 1.65 RE/d, thus indicating that these animals were absorbing nearly 83% of their daily intake of the vitamin. Efficiency of absorption in the rat has been reported to range from 37 to 48% (29–31) and recent work suggests that it may be ~60% (M. H. Green and J. B. Green, unpublished observations). Thus, based on model-derived values, rats in the present study appeared to be very efficient in their absorption of dietary vitamin A. However, the vitamin A mass values for liver, kidneys, and carcass as measured by HPLC analysis were higher in three rats killed at 12 min as compared to five rats killed at 35 d. This suggested that rats were in a negative vitamin A balance during the turnover study (i.e., 2 RE/d was not sufficient to assure that rats were in a steady state with regard to vitamin A nutriture). We assumed that if tissue masses were decreasing slowly during the course of the experimental period, the fractional rate constants for the model would remain time-invariant. The validity of this assumption needs to be tested in future studies with this same type of animal model. This might be accomplished by administering a second dose of donor plasma labeled with a different isotope (e.g., ^{14}C) approximately halfway through the experimental period and monitoring the two labels simultaneously for the remainder of the experimental period. Parallel models for both labels could be constructed and the similarity of the rate constants and utilization rates associated with each label could be compared.

An additional concern involved the quantitation of vitamin A compounds for tissues in which the vitamin A levels were so low. The need to highly concentrate the hexane–isopropanol total lipid extracts from most tissues prior to HPLC analysis resulted in the introduction of large amounts of lipid onto the HPLC column. This interfered with subsequent quantitation of both retinol and the retinyl acetate internal standard, especially for the carcass. Future studies involving the analysis of tissues containing such low concentrations of vitamin A should include a step to remove lipids that interfere with subsequent analyses. Jarnigan et al. (32) have recently reported a method that removes at least 90% of lipid from a total lipid extract prior to HPLC analysis for vitamin D compounds. A similar approach may be useful for the analysis of retinoids in vitamin A-deficient tissues. Given the problems associated with the quantitation of the low concentration of vitamin A in the carcass in the present study, it is possible that carcass vitamin A content might be more accurately estimated by the model than by HPLC analysis.

Several of the model-derived kinetic parameters associated with the liver provide further insight into hepatic metabolism of vitamin A. The average retinol molecule entering compartment 3 of the liver (Fig. 4) spent about 30 min during one passage through this compartment and the compartment turned over very rapidly (5.8 RE/d). Of the vitamin A coming into compartment 3, approximately 86% was mobilized into the plasma presumably as RBP-retinol secreted for the first time or retinol that was being recycled. Approximately 10% went to the more slowly turning-over delay component 4 and the remaining 4% entered the fecal delay and was irreversibly lost from the system. Vitamin A that entered liver component 4 had a transit time of nearly 31 h. A relatively small portion of the output from component 4 went to compartment 5 and once there turned over very slowly. We speculate that, in vitamin A-sufficient rats, one or more of these liver retinol compartments would exchange vitamin A with the large hepatic pools of retinyl esters present at higher levels of vitamin A nutriture. One possible reason that the delay element was not distinguished in an earlier study with a more vitamin A-sufficient group of rats (2) could be that samples need to be taken before one day as in the present study or that the vitamin A stores may have been masking the more subtle parts of the system. Thus, with regard to vitamin A metabolism, the underlying kinetic structure of the liver as well as other organs may be more apparent in animals that have lower levels of the vitamin.

The kidneys are one of the more active sites of vitamin A metabolism (33–35). The high level of cycling of retinol (5.5 cycles) taken up by the kidneys coupled with the high turnover rate of compartment 13 (9.0 RE/d) are indicative of the important role the kidneys play in the cycling of retinol in the system.

In contrast to rats with more sufficient vitamin A nutriture (2), the carcass, rather than the liver, had the highest level of total vitamin A in rats in the present study group. This extrahepatic vitamin A mass was located primarily in the "true" carcass since the other tissues comprising the lumped carcass (i.e., small intestine, eyes, adrenals, testes, and lungs) accounted for less than 10% of the estimated lumped carcass mass. Carcass compartment 16 is the most rapidly turning-over carcass compartment (8.2 RE/d), with molecules of vitamin A spending 5.5 h there during each transit. The remaining two carcass compartments, 17 and 24, represent larger and slower turning-over compartments, as reflected in their relatively low turnover rates (0.34 and 1.0 RE/d, respectively) and corresponding transit times (20.3 and 2.3 d). Of the retinol entering carcass compartment 16, approximately 76% was recycled back to the plasma, 12% went to compartment 24, 4% to compartment 17, and 7% was irreversibly lost by way of the carcass metabolite delay via L(6, 16).

The inclusion of urinary and fecal collection data for development of the present model provided several important advantages. These data constrained the interactions within the system relative to the major routes of metabolic loss from the system and allowed us to speculate on possible intermediary pools existing between tissue retinol pools and irreversible loss from the system. They also allowed us to quantitate the relative role of urinary versus fecal excretion of vitamin A metabolites and to identify a possible irreversible loss of vitamin A metabolites from sources other than the urine and feces. We hypothesize that one explanation for such a loss could be a release of vitamin A metabolites from the skin, perhaps as sloughed cells or secretory losses. The presence of several vitamin A compounds (e.g., retinol, dehydroretinol) in the epidermal layer of human skin has been confirmed by Vahlquist et al. (36). Further research will be needed to investigate the possible irreversible loss of vitamin A metabolites from the skin as well as other routes besides the urinary and fecal outputs. Additionally, the presence of the proposed metabolite pools will need to be verified.

It is possible to offer a few qualitative comparisons between the present model and an earlier model describing vitamin A metabolism in a more sufficient group of animals (2). In general, the low vitamin A content of the tissues and plasma of the low vitamin A status group did not inhibit the transfer of relatively large amounts of vitamin A throughout the body. The amount of total vitamin A in transit, as reflected in the plasma turnover rate, is small in relation to the liver stores in the sufficient rat. This is not the case in the low vitamin A status rat. This observation is evident by comparing the ratio of vitamin A moving through the plasma (i.e., plasma turnover rate) to the vitamin A liver stores in both groups. The ratio is essentially a measure of the number of vitamin A liver pools flowing through the plasma per unit time. For the low vitamin A status group this ratio is 18.5 d^{-1} (20.3 RE/d ÷ 1.1 RE), more than 15 times higher than the more sufficient group value of 1.2 d^{-1} (87.0 RE/d ÷ 73.3 RE). Thus, the higher ratio in the low vitamin A status group indicates that by having a much larger portion of the total liver vitamin A in transit at any one time, the rats were better able to adjust to the short-term needs of a particular tissue. This ability may be an important compensatory response to the low vitamin A content of the liver. The transit times for plasma compartment 11, liver compartment 3, and carcass compartment 16 were similar in the two groups. That is, there was a tendency for vitamin A molecules in both groups to stay in these compartments approximately the same amount of time during each passage through the compartment. In the more sufficient group, the higher levels of vitamin A in these compartments helped to account for their higher retinol turnover rates. The kidneys were unlike the plasma, liver, and carcass in that vitamin A molecules in the low vitamin A status group stay in kidney compartment 13 less time during each pass through this compartment versus that for the sufficient group. Additionally, the higher turnover rates suggest that there was more vitamin A moving through compartment 13 in this group as compared to the sufficient group. In contrast to the sufficient group, the kidneys in the low vitamin A group appeared to play a particularly active role in the turnover of vitamin A through the system. An average molecule of vitamin A cycled through kidney compartment 13 approximately 5.5 times in the low vitamin A group, whereas there was no apparent cycling to the corresponding compartment in the sufficient group. There was more cycling to the livers of the sufficient group (10.6 cycles) as compared to the low vitamin A group (3.5 cycles). The degree of cycling in the plasma and carcass compartments that could be compared was similar in the two groups. It is possible that some differences in individual tissue vitamin A kinetics, particularly for the kidney, may be a result of an expanded experimental design in the present study that included collection of tissue samples at earlier time points in the experimental period. The difference in individual tissue kinetics will have to be examined further by incorporating a similar design into future studies of more vitamin A-sufficient rats.

A high level of retinol recycling has been a consistent finding in previous kinetic studies (1–3). An earlier study suggested that vitamin A recycling to the plasma was most likely not in the form of lipoprotein-bound retinyl esters

but rather retinol associated with RBP (2). Recent work from Goodman's laboratory (37) has suggested a possible mechanism for at least a portion of the high level of retinol recycling seen in the present study as well as earlier work (1–3). Using an RBP cDNA probe, Soprano, Soprano, and Goodman (37) examined RBP mRNA levels in various tissues of rats in different states of vitamin A nutriture. They found that a wide variety of extrahepatic tissues were able to synthesize RBP and hypothesized that retinol may be recycled from extrahepatic tissues associated with RBP that is synthesized locally.

In summary, we have presented a useful animal model for studying vitamin A metabolism in rats with chronic low vitamin A status and we propose a compartmental model to describe whole-body vitamin A kinetics. Overall, the model provides evidence that these animals were capable of utilizing available vitamin A in a highly efficient manner. The data indicate that there were high levels of recycling of retinol between the plasma and tissues even in a state of very low vitamin A nutriture. Although the physiological need to move such large amounts of vitamin A through the system of rats with such low vitamin A status requires further clarification, it would appear that such an arrangement represents a high response type of system that makes it possible to quickly adjust the distribution of vitamin A in response to alterations in nutritional, metabolic, or physiological states. The presence of such a system has been hypothesized for animals with more sufficient vitamin A nutriture (2). In the low vitamin A status animal, the high response system may serve a critical function in that it represents an important compensatory mechanism to lessen the effects of vitamin A depletion. The data also suggest the possibility of a more kinetically important role for both the kidney and the carcass and a lesser role for the liver in regard to vitamin A turnover, recycling, and storage of the vitamin in rats with low vitamin A status as compared to more vitamin A-sufficient rats studied previously.

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