

# Ascorbic acid disposition kinetics in the plasma and tissues of calves

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**Toutain, P. L., D. Béchu, and M. Hidiroglou.** Ascorbic acid disposition kinetics in the plasma and tissues of calves. *Am. J. Physiol.* 273 (Regulatory Integrative Comp. Physiol. 42): R1585–R1597, 1997.—Kinetic plasma disposition parameters and tissue distribution of ascorbic acid (AA) and dihydroascorbic acid (DHA) were determined in newborn calves. After a radiolabeled AA intravenous administration, the plasma clearance (Cl) was low ( $40.8 \pm 9.5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ), the steady-state volume of distribution ( $V_{ss}$ ) was very high ( $8.9 \pm 2.2 \text{ l/kg}$ ), and the AA mean residence time (MRT) was long ( $230 \pm 85 \text{ h}$ ). After administration of a 3-g dose of AA, the Cl was high ( $450 \pm 146 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ), the  $V_{ss}$  was low ( $0.658 \pm 0.236 \text{ l/kg}$ ), and the MRT was short ( $1.49 \pm 0.41 \text{ h}$ ), indicating a strong nonlinearity of AA disposition in calves and the impossibility of preventing scurvy with the use of a loading AA dose. Nonlinearity was explained by the saturation of both kidney reabsorption and tissue uptake. The estimated AA body pool size was  $23.1 \pm 6.8 \text{ mg/kg}$ . On the basis of a compartmental analysis and actual tissue concentration measurements, it is suggested that the lung (19% of the pool) constitutes a low-capacity but rapidly mobilized pool able to cover an acute need for AA, whereas muscle and liver (40 and 33% of the pool, respectively) are high-capacity AA pools, but slowly mobilized and involved in covering the calf's long-term AA requirements. The average daily AA entry rate over the first 7 days of life was  $3.43 \pm 1.16 \text{ mg/kg}$ , and it is suggested that the calf is able to synthesize AA at an early stage.

compartmental analysis; tissue distribution; body pool size; entry rate

MAMMALIAN SPECIES, with the exception of human beings, other primates, and guinea pigs, are able to synthesize ascorbic acid (AA) in either the liver or kidney and therefore do not require dietary supplementation (18). According to this generally accepted view, the enzymatic capacity of the adult cow liver to synthesize AA is sufficient to cover vitamin C requirements. However, it was pointed out that adult cattle are prone to AA deficiency when AA synthesis is impaired because exogenous supplies of this vitamin are rapidly destroyed by the ruminal microflora (10). It was also reported that synthesis of vitamin C in calves does not occur until 2–3 wk after birth (26). Thus, during the first week after birth, the calf's AA requirements must be supplied by colostrum and milk and prenatal storage of vitamin C, which in turn relies on the mother's capacity to synthesize AA. This view is supported by the observation on scurvy-type dermatosis in calves receiving insufficient whole milk, which can be successfully treated with injections of AA. More generally, scurvy and low blood AA content in weaned calves were reported (20), suggesting that during the first week of

life calves need ~2–2.5 mg/kg of AA per day in the milk. Collectively, these observations indicate that AA status in the calf merits attention, especially from birth to the onset of endogenous ascorbate synthesis. Currently, there is little definitive information concerning vitamin C requirements in ruminants, including calves. To document these requirements, at least two physiological parameters need to be known: the plasma AA concentration considered to be nutritionally appropriate and the plasma AA clearance. The purpose of the present study was to provide basic physiological parameters characterizing AA disposition in calves, namely the plasma clearance, metabolic pool size, entry rate, tissue distribution, absorption from the digestive tract, hepatic first-pass effect, and urine elimination rate. As already reported, AA can be accumulated in blood cells and platelets (13), and special attention has been paid to the distribution of AA between plasma and blood cells.

## MATERIALS AND METHODS

### *In Vitro Evaluation of the Influx and Efflux of AA in Blood Cells*

The *in vitro* influx of AA from plasma to blood cells was examined by centrifugal method. [<sup>14</sup>C]AA [10,000 disintegrations per min (dpm)/ml] was added to aliquots of fresh heparinized calf blood and incubated at 37°C. After AA addition, the tubes were shaken continuously. Samples were taken at 0, 1, 2, 3, 4, 5, 7, 10, 20, and 30 min and 1, 2, 3, and 5 h. The samples were immediately centrifuged for 15 min at 1,000 g, and the radioactivity was measured separately in the plasma and packed cell components.

In a second experiment, the rate of AA efflux from the red blood cells was estimated as follows. A volume of calf blood was incubated with [<sup>14</sup>C]AA for 5 h at 37°C, after which the blood cells were collected after gentle centrifugation (15 min at 1,000 g). Four grams of radioactive blood cells was added to tubes containing 8 ml of control plasma and resuspended by shaking. An aliquot fraction (2 ml) was centrifuged (15 min at 1,000 g), and samples were collected at 1, 2, 3, 4, 5, 10, 15, 20, 30, 60, and 120 min. Plasma and blood cell radioactivities were measured separately as described above.

The AA activity in whole blood was calculated from the concentrations in blood cells, plasma, and from the hematocrit using the formula: total blood activity =  $0.6 \times \text{plasma activity} + 0.4 \times \text{blood cell activity}$ .

### *In Vivo Experiments*

**Animals.** Holstein calves, 1–6 days old (mean  $4.65 \pm 1.98$  days), weighing from 35 to 49 kg (mean  $44.4 \pm 4.7$  kg) at the beginning of the experiment were placed in individual cages 24 h after birth. They were fed with colostrum (AA concentrations of ~16 µg/ml) then with milk (AA concentrations of ~2

µg/ml). All calves received at least 50 µg/kg body wt of vitamin C within the first 24 h, and they were subsequently fed milk at 5% of their body weight in the morning and 5% of their body weight in the evening. The milk was collected from the cows in the dairy herd of the Animal Research Center at Greenbelt (Ottawa, ON). The calves were assigned to five groups of five animals each, corresponding to intravenous, intramuscular, intraruminal (ir), and intraperitoneal routes of <sup>14</sup>C-labeled AA administration. The fifth group of five calves received a pharmacological dose (3 g) of AA intravenously.

In a separate experiment, plasma, colostrum, and milk concentrations of AA were measured in another group of six Holstein cows and their calves at parturition and at 2, 7, 14, 21, and 28 days after calving.

#### Labeled AA

L-[Carboxyl-<sup>14</sup>C]AA was obtained from Amersham (Elk Grove, IL). The specific activity was 566 MBq/mmol (86 µCi/mg). The purity (95.8%) was checked by high-performance liquid chromatography on aminex ion exclusion HPX-87H with a gradient sulfuric acid. The dosing solution contained 10 µCi/ml in distilled water. The dose was verified by measuring the radioactivity of weighed aliquots.

#### Radiolabeled Compound Administration

The weight of the syringe was recorded before and after dosing to confirm the actual amount of administered material. All the calves received a single 50 µCi administration of AA except one (*nb 9*, intravenous route) that received only 35 µCi in error. Dose-dependent kinetic parameters for this calf were corrected to the nominal dose. For the intravenous administrations, the dose was administered via a catheter in the jugular vein; the intramuscular administration was done in the neck muscle; the intraruminal administration was done directly throughout the left flank using a needle; the intraperitoneal administration was done in the right flank.

#### Blood Sampling and Processing

Blood samples (5 ml) were obtained by direct venipuncture from the jugular vein before (control) and 1, 2, 3, 4, 5, 6, 7, 10, 24.5, 32.5, 48, 55, 72, 79, 96, 103, 120, 127, 144, 151, 168, and 175 h (7 days) postadministration of labeled AA. After the 3-g dose of AA, blood samples were collected in heparinized tubes and immediately chilled at 0, 0.1, 0.167, 0.33, 0.5, 1, 2, 3, 4, 5, 6, 7, 12, 24, 32, and 53 h after dosing. For the six calves that were sampled from birth to 28 days, blood samples were taken 6 h after birth and 1 h after the morning feeding for the other sampling days. The samples were centrifuged (5 min at 1,000 g). Two milliliters of 3.6% of metaphosphoric acid was added to 4 ml of plasma, and deproteinized plasma was stored at -70°C until analysis. The blood cells were freeze dried and kept at the laboratory temperature.

#### Urine Collection and Processing

Ottawa plastic metabolism cages (21), molded from fiberglass and equipped with a modified device for separation of feces and urine, were used for urine collection, which was collected at 4°C. The total amount of individual daily urine was mixed inside the collection bottle and a 100-ml sample was collected for analysis.

#### Animal Euthanasia, Tissue Sampling, and Processing

After completion of the blood sampling (7 days postadministration) all animals were killed with the use of a captive-bolt pistol and exsanguination. Tissues and organs were rapidly

dissected, dried with a filter paper, and coarsely chopped before storage at -70°C before radioassay.

The following samples were collected for measurement of radioactivity: muscle (hip, neck), fat (perirenal), liver, kidney, spleen, heart, lung, pancreas, and adrenal glands.

#### Analytic Methods

Specific activity is the sum of AA and dihydroascorbic acid (DHA) activities. Because 95% of the radioactivity was related to AA, the results are reported in terms of AA.

Triplicate tissue (50–100 mg) samples from each organ were placed in ashless cellulose pellets (400 mg) and were burned in sample oxidizer (Packard Instrument, Downers Grove, IL; model 306). Radioactivity was performed by a liquid scintillation spectrometer (model 2/5–250 Beckman, Fullerton, CA).

Chemical analysis for vitamin C was initiated within 1 or 2 days after the animal was killed. AA and DHA concentrations in samples were measured by high-performance liquid chromatography using an electrochemical detector as extensively described elsewhere (3, 4). The level of quantification for AA was 0.5 µg/ml, and the interassay variation was below 7%.

#### Pharmacokinetic Analysis

After each administration, activity versus time was measured in freeze-dried blood cells and plasma. The results for the freeze-dried blood cells were expressed on a dry matter basis. On the assumption that the dry matter content is 33% of the red blood cell mass (12), a water blood cell activity equal to plasma activity, and a mean hematocrit of 40%, the total blood activity (TBA) was approximated with Eq. 1

$$\text{TBA} = 0.13 \times \text{freeze-dried blood cell activity} + 0.87 \text{ plasma activity} \quad (1)$$

The AA did not accumulate in the blood cells, and all in vivo kinetic analyses were performed using only plasma activity with a program for nonlinear regression analysis adapted from the MULTI analysis program (37). Plasma activity (dpm/ml) was fitted to the general polyexponential Eq. 2

$$A(t) = \sum_{i=1}^n Y_i \exp(-\lambda_i t) \quad (2)$$

where  $A(t)$  is the plasma activity (dpm/ml) at time  $t$  (h),  $Y_i$  (dpm/ml) is the coefficient of the  $i^{\text{th}}$  exponential term, and  $\lambda_i$  ( $\text{h}^{-1}$ ) is the  $i^{\text{th}}$  exponential decay term. Initial estimates were obtained using the residual method (15). The data points were weighed by the inverse of the squared-fitted value ( $1/\hat{Y}^2$ ). The best fit was obtained by minimizing the weighed least-square criteria, and the number of exponents (1, 2, or 3) needed for each data set was determined by application of the Akaike's information criterion (36). On the basis of this criterion, a triexponential equation was selected for the intravenous administration

$$A(t) = Y_1 \exp(-\lambda_1 t) + Y_2 \exp(-\lambda_2 t) + Y_3 \exp(-\lambda_3 t) \quad (3)$$

where  $Y_1$ ,  $Y_2$ , and  $Y_3$  (dpm/ml) are preexponential coefficients and  $\lambda_1$ ,  $\lambda_2$ , and  $\lambda_3$  ( $\text{h}^{-1}$ ) are exponents. Data were therefore interpreted using a three-compartment open model with activity elimination from the central compartment.

The estimated parameters ( $Y_1$ ,  $Y_2$ ,  $Y_3$  and  $\lambda_1$ ,  $\lambda_2$ ,  $\lambda_3$ ) were used to solve the first-order rate constants of transfer from central to peripheral compartments ( $K_{21}$ ,  $K_{31}$ ,  $K_{12}$ ,  $K_{13}$ ) with classical equations (15).

The volume of the central compartment (ml/kg) was obtained with

$$V_c = \text{dose}/(Y_1 + Y_2 + Y_3) \quad (4)$$

where dose is the administered dose ( $111 \times 10^6$  dpm).

The steady-state volume of distribution ( $V_{ss}$ ) (ml/kg), which is the appropriate volume to consider when determining the amount of AA in the body at equilibrium, was obtained with Eq. 5

$$V_{ss} = V_c [1 + (K_{21}/K_{12}) + K_{31}/K_{13}] \quad (5)$$

where  $V_c$  is the volume of the central compartment, as given in Eq. 4, and  $K_{21}$  and  $K_{31}$  are the first-order rate constants of transfer from the central compartment to peripheral compartments 2 and 3, respectively, and  $K_{12}$  and  $K_{13}$  are the first-order rate constants of transfer from peripheral compartments 2 and 3 to the central compartment.

$V_{\text{area}}$  (ml/kg) is the appropriate volume to consider for calculating the amount of labeled AA remaining at the time of death, i.e., when the pseudodistribution equilibrium has been reached. After intravenous administration,  $V_{\text{area}}$  was obtained using Eq. 6

$$V_{\text{area}} = \text{dose}/(\text{AUC} \times \lambda_3) \quad (6)$$

where  $\lambda_3$  is the slope of the terminal phase and AUC ( $\text{dpm} \cdot \text{h}^{-1} \cdot \text{ml}^{-1}$ ) is the area under the plasma activity curve obtained by integrating Eq. 3, i.e.

$$\text{AUC} = Y_1/\lambda_1 + Y_2/\lambda_2 + Y_3/\lambda_3 \quad (7)$$

The plasma clearance (Cl) ( $\text{ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) was calculated using

$$\text{Cl} = \text{dose}/\text{AUC} \quad (8)$$

Clearance was also calculated using the trapezoidal rule with extrapolation to infinity. The distribution clearances that characterize the rate of exit of AA from the central compartment to the first and second peripheral compartments were obtained with Eqs. 9 and 10, respectively

$$\text{Cld}_{21} = K_{21} \times V_c \quad (9)$$

and

$$\text{Cld}_{31} = K_{31} \times V_c \quad (10)$$

where  $\text{Cld}_{21}$  is the distribution clearance to peripheral compartment 2,  $\text{Cld}_{31}$  is the distribution clearance to the peripheral compartment 3; and  $V_c$  is as obtained with Eq. 4.

The AA renal clearance (Clr) (l/h) was obtained for calves treated by intravenous and intramuscular routes. Clr was obtained with Eq. 11

$$\text{Clr} = \text{activity eliminated by urine (0-7 days)} / \text{AUC (0-7 days)} \quad (11)$$

where activity eliminated by urine (0-7 days) is the total activity collected in urine during the 7 days after labeled AA administration and AUC (0-7 days) is calculated by the linear trapezoidal rule.

The terminal plasma half-life ( $t_{1/2}$ ) (h) after intravenous administration was obtained using Eq. 12

$$t_{1/2} = \ln(2)/\lambda_3 \quad (12)$$

Different MRT and mean transit times (MTT) were calculated (23, 32, 33).

After intravenous administration, the MRT of the activity in the system, i.e., the mean total time taken for a labeled molecule to transit through the body, was calculated with Eq. 13

$$\text{MRT} = \frac{Y_1/\lambda_1^2 + Y_2/\lambda_2^2 + Y_3/\lambda_3^2}{Y_1/\lambda_1 + Y_2/\lambda_2 + Y_3/\lambda_3} \quad (13)$$

After intravenous administration, the MRT in the central compartment ( $\text{MRT}_c$ ), i.e., the average total interval of time spent by the molecule in the central compartment in all its passages through it, was obtained with Eq. 14

$$\text{MRT}_c = 1/K_{01} \quad (14)$$

where  $K_{01}$  is the first-order rate constant of elimination from the central compartment.

The MRT in the peripheral compartments ( $\text{MRT}_T$ ), i.e., the average interval of time spent by a molecule in the two peripheral compartments in all of its passages, i.e.,  $R$  passages (see Eq. 19) was obtained with Eq. 15

$$\text{MRT}_T = \text{MRT} - \text{MRT}_c \quad (15)$$

where MRT was obtained using Eq. 13 and  $\text{MRT}_c$  was obtained using Eq. 14.

The  $\text{MTT}_c$ , i.e., the average interval of time spent by a molecule of AA from its entry into the central compartment to its next exit, was obtained with Eq. 16

$$\text{MTT}_c = 1/(K_{01} + K_{21} + K_{31}) \quad (16)$$

The MTT in the first (shallow) peripheral compartment ( $\text{MTT}_{p1}$ ), i.e., the average interval of time spent by a molecule of AA from its entry into the first peripheral compartment to its next exit, was obtained with Eq. 17

$$\text{MTT}_{p1} = 1/K_{12} \quad (17)$$

The MTT in the second peripheral (deep) compartment ( $\text{MTT}_{p2}$ ), i.e., the average interval of time spent by a molecule of AA from its entry into the second peripheral compartment to its next exit, was obtained with Eq. 18

$$\text{MTT}_{p2} = 1/K_{13} \quad (18)$$

The number of cycles around the central compartment ( $R$ ), i.e., the average number of times a molecule returns to the central compartment after a passage through it, was obtained with Eq. 19

$$R = (K_{21} + K_{31})/K_{01} \quad (19)$$

The  $\text{MTT}_T$ , i.e., the average interval of time spent by a molecule from its entry into one of the two peripheral compartments to its next exit, was obtained with Eq. 20

$$\text{MTT}_T = \text{MRT}_T/R \quad (20)$$

where  $\text{MRT}_T$  was obtained in Eq. 15 and  $R$  in Eq. 19.

At steady state, the time necessary to have a 50% drop in plasma concentration or amount in the body (5) if the entry rate is totally stopped is given in Eq. 21 and 22, respectively

$$t_{1/2} \text{ plasma mean drop} = 0.693 \text{ MRT}_c \quad (21)$$

$$t_{1/2} \text{ amount mean drop} = 0.693 \text{ MRT} \quad (22)$$



On the basis of Akaike's information criteria (36), the individual plasma activity obtained after intramuscular, intraruminal, and intraperitoneal AA administrations were best fitted with Eq. 23 or 24

$$A(t) = -(Y_1 + Y_2) \exp(-K_a t) + Y_1 \exp(-\lambda_1 t) + Y_2 \exp(-\lambda_2 t) \quad (23)$$

$$A(t) = -(Y_1 + Y_2 + Y_3) \exp(-K_a t) + Y_1 \exp(-\lambda_1 t) + Y_2 \exp(-\lambda_2 t) + Y_3 \exp(-\lambda_3 t) \quad (24)$$

where  $K_a$  ( $\text{h}^{-1}$ ) is the apparent first-order rate constant of absorption. The data were therefore described by the two- or three-compartment model with a single process of absorption.

The  $t$  of the terminal phase was calculated with Eq. 25

$$t_{\%} = \ln(2)/\lambda_{2 \text{ or } 3} \quad (25)$$

$T_{\text{max}}$ , which is the time corresponding to the occurrence of the maximal plasma activity ( $C_{\text{max}}$ ) and  $C_{\text{max}}$  were calculated from Eq. 23 with the classical equation (15) or by solving Eq. 24.

The apparent systemic availabilities (F%) of AA after intramuscular, intraruminal, and intraperitoneal administrations were calculated with Eq. 26

$$F\% = \frac{\text{AUC (im, ir, or ip)}}{\text{AUC (iv)}} \times \frac{\text{dose iv}}{\text{dose (im, ir, or ip)}} \times 100 \quad (26)$$

where dose is the administered dose per body weight unit for the corresponding group of five calves. AUC were calculated using the trapezoidal rule with extrapolation to infinity.

The average entry rate of AA ( $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) was calculated for the five calves administered by intravenous route with Eq. 27

$$\text{Entry rate} = \text{Cl} \times \text{AA}_{\text{plasma concn}} \quad (27)$$

where Cl is calculated using Eq. 8 and  $\text{AA}_{\text{plasma concn}}$  is the overall mean AA plasma concentration calculated over the 7 sampling days using the trapezoidal rule.

The average AA pool size (mg/kg) of the five calves administered by intravenous route was obtained with Eq. 28

$$\text{AA}_{\text{pool size}} = V_{\text{ss}} \times \text{AA}_{\text{plasma concn}} \quad (28)$$

The AA pool size of five calves administered by the intravenous route was also calculated at the time of death with the use of Eq. 28, but replacing the overall mean  $\text{AA}_{\text{plasma concn}}$  with the plasma  $\text{AA}_{\text{plasma concn}}$  actually measured at the time of death. In addition, the amount of AA ( $\mu\text{g}/\text{kg}$ ) in each of the three compartments at the time of death time was obtained with Eqs. 29, 30, and 31

$$\text{Amount}_{\text{cc}} = V_{\text{c}} \times \text{AA}_{\text{plasma concn}} \quad (29)$$

$$\text{Amount}_{\text{p1}} = \frac{V_{\text{c}} \times K_{21}}{K_{12}} \times \text{AA}_{\text{plasma concn}} \quad (30)$$

$$\text{Amount}_{\text{p2}} = \frac{V_{\text{c}} \times K_{31}}{K_{13}} \times \text{AA}_{\text{plasma concn}} \quad (31)$$

where  $\text{Amount}_{\text{cc}}$  is the amount in the central compartment,  $\text{Amount}_{\text{p1}}$  is the amount in the first peripheral compartment, and  $\text{Amount}_{\text{p2}}$  is the amount in the second peripheral compartment.

After the intravenous administration, the labeled AA remaining at the time of death (7 days) was calculated with Eq. 32

$$\text{Remaining activity} = V_{\text{area}} \times \text{AA}_{\text{activity}} \quad (32)$$

where  $\text{AA}_{\text{activity}}$  is the measured plasma activity at the time the animals were slaughtered.

Plasma AA concentrations ( $\mu\text{g}/\text{ml}$ ) after the intravenous AA administration at the pharmacological dose (3 g) were analyzed with the use of the same approach as plasma activity. The presence of physiological concentrations of AA was taken into account and based on the Akaike's information criterion. A biexponential equation corresponding to a bicompartamental model was selected

$$C(t) = Y_1 \exp(-\lambda_1 t) + Y_2 \exp(-\lambda_2 t) + \text{base} \quad (33)$$

where  $C(t)$  is the plasma AA concentration at time  $t$  and base is the control AA plasma concentration. Other parameters (Cl,  $V_{\text{ss}}$ , MRT) were calculated as for the plasma activity.

### Statistical Analysis

Statistical analysis was performed using STATGRAPHICS (STSC, Rockville, MD). Values are reported as means  $\pm$  SD. The effect of time (independent variable) over the in vitro plasma and blood cell activity and blood-to-plasma ratio was studied using linear regression. Analysis of variance (ANOVA) for repeated measures was performed. Homogeneity of variance was tested with the use of Bartlett's test. Nonparametric ANOVA (Friedman 2-way analysis) was carried out when the variances were not homogeneous.

## RESULTS

### In Vitro Blood Distribution Studies

When fresh blood was spiked with radiolabeled AA, plasma and blood cell activities remained essentially constant for incubation times ranging from 0 to 5 h (regression analysis,  $P > 0.05$ ) (Fig. 1A), indicating an immediate distribution of AA between plasma and blood cells; the mean  $\pm$  SD AA blood-to-plasma ratio was  $0.71 \pm 0.03$ .

In the second experiment, the efflux of AA from blood cells was evaluated by adding blood cells incubated with labeled AA to control plasma; in this experiment, there was no influence of time (from 0 to 120 min) on the blood-to-plasma ratio (regression analysis,  $P > 0.05$ ) (Fig. 1B). The mean  $\pm$  SD ratio was  $0.99 \pm 0.06$ .

These results indicate that under in vitro conditions there was no accumulation of AA in blood cells, and the distribution of AA between blood cells and plasma was very rapid.

### In Vivo Distribution of AA Between Plasma and Blood Cells

Figure 2 shows the effect of time on AA activity in plasma, freeze-dried blood cells, and total blood for a representative calf after an intravenous administration of AA. Inspection of Fig. 2 indicates that AA plasma and total blood activities were similar at the first sampling time but that blood activities became progressively higher than the corresponding plasma activities

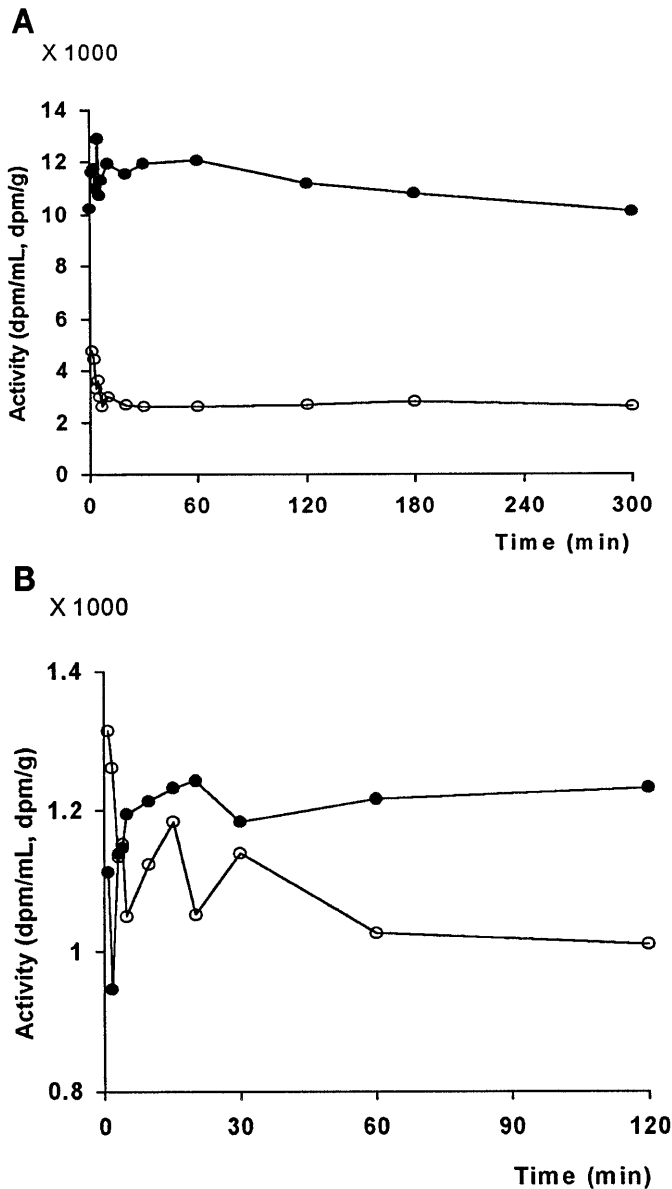


Fig. 1. A: plasma (●) activity [disintegrations/min (dpm)/ml] and red blood cell (○) activity (dpm/g) after adding 10,000 dpm/ml [<sup>14</sup>C]ascorbic acid to fresh whole blood. B: plasma (●) activity (dpm/ml) and red blood cell (○) activity (dpm/g) after adding 8.0 ml of control plasma in 4 g of red blood cells incubated with [<sup>14</sup>C]ascorbic acid.

until 10 h postadministration. After 10 h, total blood and plasma AA activities decreased in parallel.

Figure 3 shows the mean ratio of blood to plasma activity for the five calves administered AA by the intravenous route. In steady-state conditions, the total blood-to-plasma activity ratio was between 2.2 and 2.8. After the intramuscular administration, the mean steady-state ratio was 1.6 and 1.8 at 10 and 15 h postadministration, respectively. Similar results were obtained for the intraruminal and intraperitoneal routes of administration. Because the total blood activities and plasma activities were of the same order of magnitude and, as in vivo, the equilibration rate of AA between blood cells and plasma was relatively slow, it

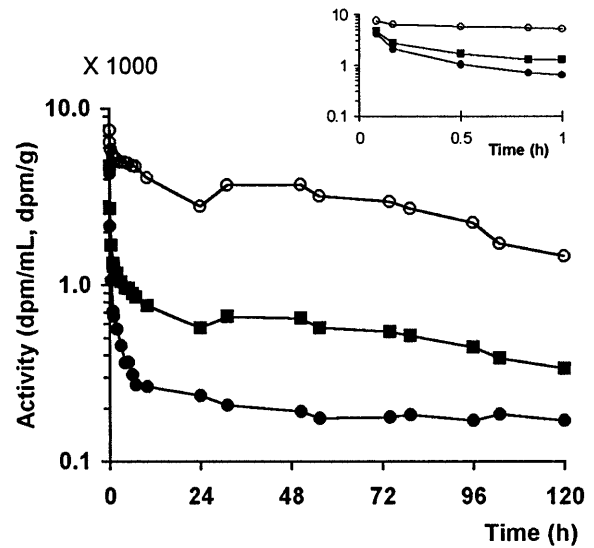


Fig. 2. Semilogarithmic plot of plasma activity (dpm/ml) (●), freeze-dried blood cell activity (dpm/g) (○), and total blood activity (dpm/ml) (■) vs. time (h) after intravenous administration of [<sup>14</sup>C]ascorbic acid (50 μCi) to a representative calf. Kinetics for 1st h are shown in inset.

was decided to perform kinetic analyses on the plasma data only (see DISCUSSION).

*Kinetic Analysis of Plasma Activity After Intravenous Administration*

The semilogarithm plot of the observed and fitted activity (dpm/ml) versus time (h) after an intravenous administration of labeled AA is shown in Fig. 4 for a representative calf. The plasma clearance was low ( $40.8 \pm 9.5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ), but the distribution clearances were high ( $1,900 \pm 1,038 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  for  $\text{Cl}_{d1}$  and  $528 \pm 155 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  for  $\text{Cl}_{d2}$ ). The  $V_{ss}$  was very high ( $8.9 \pm 2.2 \text{ l/kg}$ ). The MRT in the body ( $230 \pm 85 \text{ h}$ ) and the  $t_{1/2}$  ( $152 \pm 57 \text{ h}$ ) were prolonged.  $\text{MRT}_c$  (the MRT in the central compartment) was short ( $8.4 \pm 2.2 \text{ h}$ ) and  $\text{MRT}_T$  [the MRT in the peripheral compartments (tis-

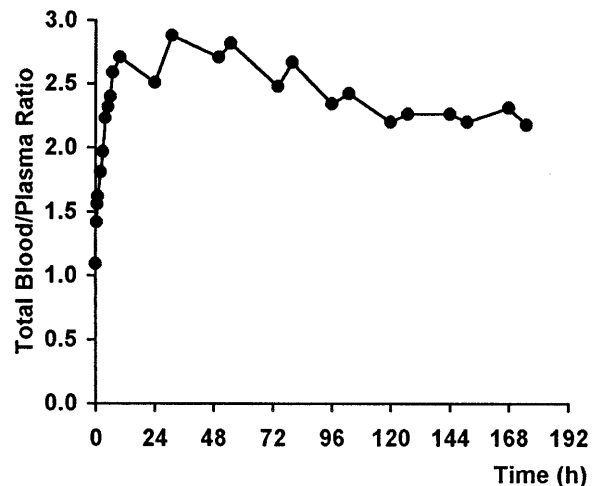


Fig. 3. Blood-to-plasma activity ratio for 5 calves administered with labeled ascorbic acid by intravenous route.

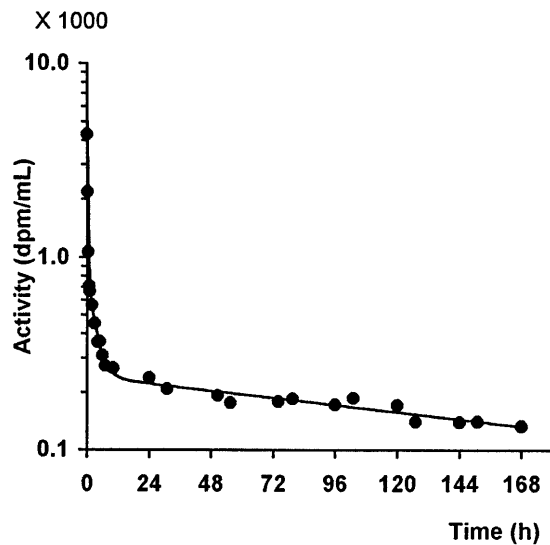


Fig. 4. Semilogarithmic plot of observed (●) and fitted (solid line) plasma ascorbic acid activity (dpm/ml) vs. time (h) after an intravenous administration of [ $^{14}\text{C}$ ]ascorbic acid (50  $\mu\text{Ci}$ ) to a representative calf.

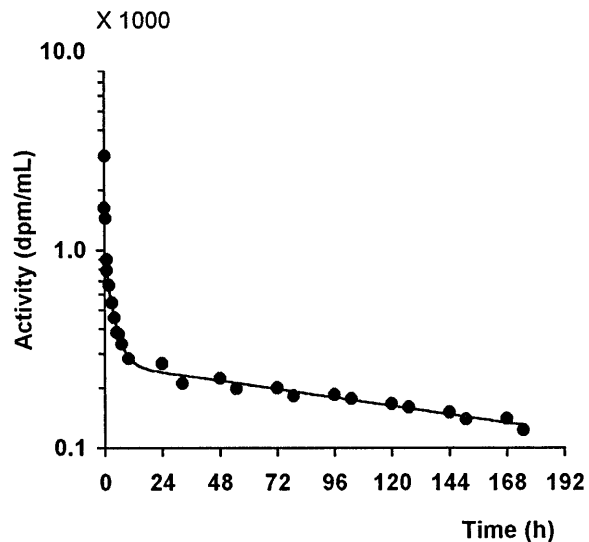


Fig. 5. Semilogarithmic plot of observed (●) and fitted (solid line) plasma ascorbic acid activity (dpm/ml) vs. time (h) after an intramuscular administration of [ $^{14}\text{C}$ ]ascorbic acid (50  $\mu\text{Ci}$ ) to a representative calf.

sue] was prolonged ( $222 \pm 84$  h). The recycling number ( $R$ ) was high ( $66 \pm 44$  per h). The  $\text{MTT}_c$  (see Eq. 16) was very short ( $11.2 \pm 8.1$  min) and the  $\text{MTT}_T$  in the peripheral compartments (see Eq. 20) was much longer ( $4.3 \pm 2.1$  h). The  $\text{MTT}_{p2}$  in the first peripheral compartment (see Eq. 17) was short ( $0.71 \pm 0.20$  h) compared with the  $\text{MTT}_{p1}$  in the second peripheral compartment ( $17.5 \pm 5.5$  h) (see Eq. 18). In steady-state conditions, the time necessary to obtain a 50% drop in plasma concentration would only be 5.8 h if entry rate was totally stopped, whereas in the same situation the time necessary to obtain a 50% drop in the AA amount in the body would be 6.64 days.

#### Kinetic Analysis of Plasma Activity After Intramuscular, Intraruminal, and Intraperitoneal Administrations

The semilogarithmic plots of the plasma activity (dpm/ml) versus time (h) for the intramuscular, intraruminal, and intraperitoneal administrations are shown for a representative calf in Figs. 5, 6, and 7, respectively.

After intramuscular administration, plasma activity was best fitted with Eq. 24. The plasma activity increased rapidly to reach a maximum value ( $2,077 \pm 225$  dpm/ml)  $0.25 \pm 0.01$  h after labeled AA administration. The apparent half-life of absorption was  $0.12 \pm 0.02$  h and the apparent  $t_{1/2}$  was  $195 \pm 17$  h. The MRT was  $270 \pm 23$  h and the apparent mean bioavailability was slightly higher than 100%.

After intraruminal administration, plasma activity was best fitted with Eq. 23. Plasma activity increased rapidly to reach a maximal value ( $1,001 \pm 180$  dpm/ml)  $0.75 \pm 0.17$  h after labeled AA administration. The apparent absorption half-life was  $0.16 \pm 0.06$  h and the  $t_{1/2}$  was  $120 \pm 33$  h. The MRT was  $168 \pm 40$  h. The apparent mean bioavailability was 62%.

After intraperitoneal administration, the plasma activity was best fitted with Eq. 23. The plasma activity increased rapidly to reach a maximum value ( $1,122 \pm 325$  dpm/ml)  $0.45 \pm 0.55$  h after labeled AA administration. The half-life of absorption was  $0.13 \pm 0.18$  h, the  $t_{1/2}$   $180 \pm 24$  h, and the MRT was  $257 \pm 31$  h. The apparent mean bioavailability was 89%.

#### Renal Clearance of AA After Intravenous and Intramuscular Administration

The total radioactivity collected in urine over the 7 days after intravenous AA administration was  $11.4 \pm 3.4\%$  of the administered dose of AA. The AA renal clearance was  $8.9 \pm 3.4$   $\text{ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ , i.e.,  $19.9 \pm 5.5\%$

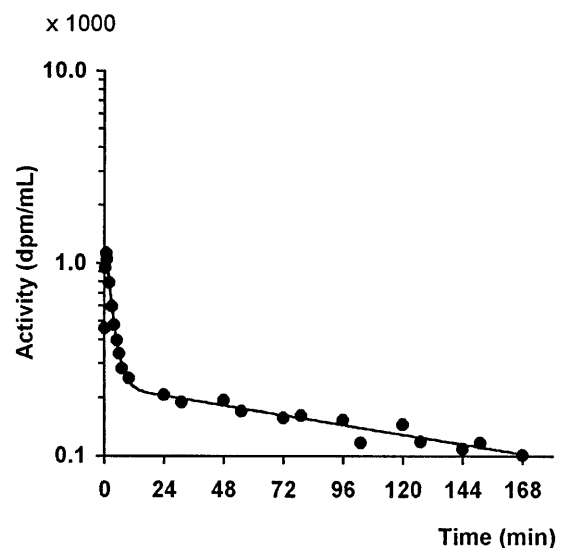


Fig. 6. Semilogarithmic plot of observed (●) and fitted (solid line) plasma ascorbic acid activity (dpm/ml) vs. time (h) after an intraruminal administration of [ $^{14}\text{C}$ ]ascorbic acid (50  $\mu\text{Ci}$ ) to a representative calf.

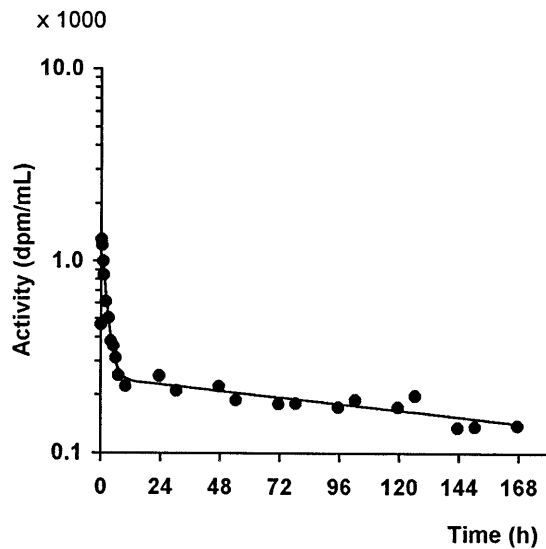


Fig. 7. Semilogarithmic plot of observed (●) and fitted (solid line) plasma ascorbic acid activity (dpm/ml) vs. time (h) after an intraperitoneal administration of [<sup>14</sup>C]ascorbic acid (50 μCi) to a representative calf.

of the plasma clearance as calculated with the trapezoidal rule. After intramuscular administration,  $13.4 \pm 3.7\%$  of the administered activity was collected within the 7 days of sampling and the estimated AA renal clearance was  $10.3 \pm 2.6 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ , i.e., a value close to that calculated after the intravenous administration.

*AA and DHA Plasma Concentrations and AA Pool Sizes (Intravenous Study)*

The mean plasma concentration of AA (from injection to 7 days postadministration) was  $3.45 \pm 0.59 \text{ μg/ml}$  for the five calves undergoing intravenous administration. The corresponding mean plasma DHA concentration was  $0.36 \pm 0.14 \text{ μg/ml}$ , indicating that DHA represented only  $9.44 \pm 3.69\%$  of the total plasma vitamin C (AA plus DHA) concentrations. Similar results were obtained with calves administered by the intramuscular, intraruminal, or intraperitoneal routes. The average AA pool size over the 7 days of sampling was  $30.0 \pm 6.6 \text{ mg/kg}$  and the total vitamin C pool size (AA plus DHA) was  $33.2 \pm 7.7 \text{ mg/kg}$ . When considering the plasma AA concentration at the time of death ( $2.60 \pm 0.41 \text{ μg/ml}$ ), the estimated body AA pool size was  $23.1 \pm 6.8 \text{ mg/kg}$  and the AA plus DHA pool size was  $26.1 \pm 8.4 \text{ mg/kg}$ . At the time of death, the amounts of AA were  $0.87 \pm 0.27 \text{ mg/kg}$  in the central compartment,  $3.1 \pm 1.5 \text{ mg/kg}$  in the first peripheral compartment, and  $19.1 \pm 5.7 \text{ mg/kg}$  in the second peripheral compartment.

*AA Entry Rate (Intravenous Study)*

The average AA entry rate over the first 7 days was  $143 \pm 48 \text{ μg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ , i.e.,  $3.43 \pm 1.16 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ . The total vitamin C entry rate (i.e., AA plus DHA) was  $3.79 \pm 1.27 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ . When considering the plasma AA concentration at death, the AA entry rate was  $2.54 \pm 0.68 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ .

*Tissue Distribution of Radioactivity (Intravenous Study)*

The tissue distribution of radioactivity 7 days after intravenous labeled AA administration is given in Table 1. The activity in all the tissues was higher than in plasma. The adrenal gland had the highest activity (131× the plasma activity). The lowest AA activities were found in adipose tissue and muscle (~4× the plasma activity).

The activity remaining in the body at slaughter was estimated from the last measured plasma activity ( $120 \pm 20 \text{ dpm/ml}$ ) and the  $V_{\text{area}}$  ( $9,408 \pm 2,180 \text{ ml/kg}$ ) to be  $51.6 \times 10^6 \text{ dpm}$ , i.e., 46.5% of the administered dose ( $111 \times 10^6 \text{ dpm}$ ).

On the basis of tissue weight for a 48-kg body wt calf (17), it was estimated that the total activity remaining at the time of death for the different tissues that were actually measured was  $23.9 \times 10^6 \text{ dpm}$ , i.e., 21.7% of the administered radioactivity ( $111 \times 10^6 \text{ dpm}$ ), suggesting that the tissues that were not measured (bone, brain, digestive tract, and skin) accounted for about one-half of the total remaining activity. Similar results were obtained with animals treated by the intramuscular route where the activity remaining in the measured tissues corresponded to 22.1% of the administered dose.

*Tissue Distribution of AA and DHA (Intravenous Study)*

Tissue concentrations of AA, DHA, and total AA concentrations at the time of death are given in Table 2. The AA concentration in all tissues was higher than in the plasma. The highest concentration was found in the adrenal gland ( $1,139 \text{ μg/g}$ , i.e., 473 times the plasma concentration) and the lowest in adipose tissue and

Table 1. Recovery and distribution of radioactivity 7 days after intravenous [<sup>14</sup>C]ascorbic acid administration

Tissue	Radioactivity in Wet Tissue (dpm/g, dpm/ml)	Tissue Wt, g	Total Radioactivity in Different Tissues, dpm × 10	Percentage of Administered Dose
Spleen	5,058 ± 503	100	506	0.46
Liver	3,545 ± 404	1,300	4,609	4.2
Kidney	1,977 ± 319	200	395	0.36
Heart	1,141 ± 247	375	428	0.39
Lung	4,779 ± 842	800	3,823	3.5
Pancreas	3,189 ± 841		ND	ND
Adipose	451 ± 118	1,000	451	0.41
Muscle (neck)	520 ± 117	23,000	11,960	10.8
Adrenal	15,695 ± 2,473		ND	ND
Blood	579 ± 101	3,000	1,737	1.6
Plasma	120 ± 20			
Skin	ND	4,500	ND	ND
Digestive tract	ND	6,000	ND	ND
Bone	ND	8,000	ND	ND
Total		48,275	23,909	21.7

Values are means ± SD (n = 5 calves). Dose of [<sup>14</sup>C]ascorbic acid 50 μCi ( $111 \times 10^6 \text{ dpm/calf}$ ). Tissue weights were obtained from literature for a 48-kg calf (17). For calf receiving a dose of 35 μCi, radioactivity in tissue was corrected to be expressed as a 50 μCi dose equivalent. ND, not determined.



Table 2. Tissue concentration of AA and DHA in calves

Tissue	AA	DHA	Total (AA + DHA)	DHA, % of total
Spleen	173 ± 27	57 ± 13	230 ± 23	24.9 ± 6.01
Liver	234 ± 51	22 ± 4.7	256 ± 53	9.0 ± 2.1
Kidney	95 ± 11.5	22 ± 4.5	117 ± 14	18.7 ± 2.9
Heart	35 ± 3.4	11.1 ± 4.3	47 ± 3.0	23.7 ± 8.6
Lung	215 ± 67	44 ± 13	259 ± 74	17.4 ± 4.9
Pancreas	126 ± 17	17.3 ± 7.4	143 ± 13.4	12.3 ± 5.6
Muscle (neck)	15.7 ± 2.9	5.0 ± 1.5	21 ± 4.0	24.1 ± 4.2
Adipose	21 ± 5.3	7.5 ± 2.7	29 ± 5.1	26.7 ± 9.3
Adrenal	1,139 ± 61	53 ± 24	1,192 ± 146	4.4 ± 1.8
Plasma	2.61 ± 0.41	0.32 ± 0.14	2.93 ± 0.49	10.8 ± 3.6

Values are means ± SD in µg/g. AA, ascorbic acid; DHA, dihydroascorbic acid.

muscle (15–20 µg/g, i.e., 6–8 times the plasma concentration).

The highest concentrations of DHA were found in the adrenal gland (53 µg/g) and spleen (57 µg/g) (~170× the DHA plasma concentration) and the lowest in muscle and adipose tissue (~20× the plasma concentration). When total vitamin C concentration was considered, the respective contributions of AA and DHA concentration varied significantly between tissues; spleen, muscle, adipose, lung, heart, and kidney had ~15–25% of the total vitamin C as DHA, and adrenal gland had only 4% of the total as DHA. Plasma and liver had intermediate levels of ~10% of the total as DHA.

#### Evaluation of the Body Pool Size of AA From AA Tissue Concentrations

The estimated amount of AA was based on the tissue weight (17) and the actual AA tissue concentration (Table 3). The total amount of AA obtained from all tissues measured was 909 mg or 18.9 mg/kg body wt. Three tissues accounted for most of the AA. They were muscle (40%), liver (33%), and lung (19%).

Table 3. Total AA and DHA body pool sizes obtained from the actual mean tissue concentrations of AA and DHA in calves of the intravenous study

Tissues	Tissue Wt, g	AA, mg	DHA, mg	Total (AA + DHA), mg
Spleen	100	17	5.7	23
Liver	1,300	304	29	333
Kidney	200	19	4.4	23
Heart	375	13	4.2	17
Lung	800	172	35	207
Pancreas	ND	ND	ND	ND
Adipose	1,000	21	7.5	28.5
Muscle (neck)	23,000	362	116	478
Adrenal	ND	ND	ND	ND
Other tissues (skin, bone, blood)		ND	ND	ND
Total		909	202	1,111

Weights of different tissues are from Ref. 17 for a 48-kg body wt calf. Using the kinetic approach, the total body pool size for AA was estimated to be 23.1 ± 6.8 mg/kg; using the kinetic approach, the total body pool size for AA + DHA was estimated to be 26.1 ± 8.4 mg/kg. See Table 1 for more information.

With the use of plasma kinetic parameters, the estimated size of the pool of AA at slaughter was 23.1 ± 6.8 mg/kg (see above), suggesting that the tissues not actually measured (skin, digestive tract, bone) contained some amount of AA.

From tissue measurements, the total pool size (AA + DHA) actually measured was 1,111 mg, i.e., 23.1 vs. 26.1 ± 8.4 mg/kg using the plasma kinetic approach.

The estimated total pool size was 26.0 mg/kg when the actual tissue concentration of AA and DHA was measured in five calves in the intramuscular study, i.e., very similar to that obtained in the intravenous study.

Tissue distribution of AA, DHA, and radioactivity after intraruminal and intraperitoneal administration followed the same pattern as after the intravenous or intramuscular administration.

#### Plasma AA, DHA, and Total Vitamin C Concentrations in the Four Groups of Calves Subjected to Radiolabeled Administration

There was a progressive decrease of AA plasma concentrations over the 7 days of the experiment. The plasma AA concentration was 4.39 ± 1.91 µg/ml for 1-day-old calves, 3.51 ± 0.70 µg/ml for 3-day-old, and 3.14 ± 0.63 µg/ml for 7-day-old calves (ANOVA,  $P < 0.01$ ). The calf group (intravenous, intramuscular, intraruminal, or intraperitoneal) had no influence on plasma AA concentration ( $P > 0.05$ ).

The DHA decreased from day 1 to day 3 or 7 (0.40 ± 0.24, 0.30 ± 0.11, and 0.29 ± 0.12 µg/ml) but the difference was not statistically significant (ANOVA,  $P > 0.05$ ).

#### AA Concentrations in Plasma and Milk in a Group of Six Calves and Their Dams

Figure 8 shows plasma AA in a group of six calves for the first 28 days of life. The AA plasma concentration declined in the first 3 wk of life, with a mean concentration of 7.65 ± 1.73 µg/ml at birth, 3.47 ± 0.75 µg/ml at 1

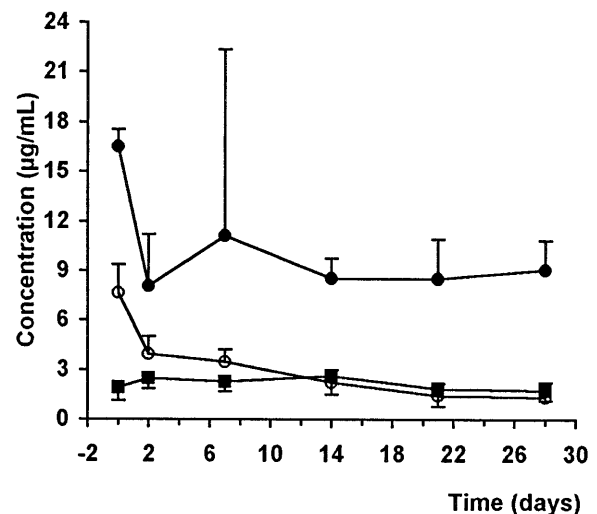


Fig. 8. Plasma (○, ■) and milk (●) concentrations (µg/ml) of ascorbic acid (mean ± SD) vs. time (days) in a group of 6 calves (○) and their dam (■, ●) during the first 28 days after calving.



wk,  $2.23 \pm 0.72 \mu\text{g/ml}$  at 2 wk, and  $1.35 \pm 0.20 \mu\text{g/ml}$  at 3 wk. The plasma AA level of cows is shown in Fig. 8; it was low and fluctuated between 1.7 and 2.6  $\mu\text{g/ml}$ . Figure 8 shows the AA in milk: the level was high in colostrum ( $16 \pm 1.02 \mu\text{g/ml}$ ) at calving, dropped sharply on day 2 ( $8.0 \pm 1.31 \mu\text{g/ml}$ ), then stabilized until 28 days postcalving ( $9.1 \pm 1.8 \mu\text{g/ml}$ ).

#### *Kinetic Disposition Parameters After an Intravenous Pharmacological Dose of AA*

The semilogarithmic plot of plasma concentration ( $\mu\text{g/ml}$ ) versus time after the intravenous administration of AA at a pharmacological dose (3 g) is shown for a representative calf in Fig. 9.

Comparison with Fig. 4 indicates clearly that elimination of AA was much more rapid after the administration of a pharmacological dose of AA than after the administration of an AA tracer dose; the plasma pharmacological clearance was  $450 \pm 146 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ , i.e.,  $\sim 11$  times higher than the physiological clearance.  $V_{ss}$  was  $658 \pm 236 \text{ ml/kg}$ , i.e., 14 times lower than the physiological  $V_{ss}$ . MRT was  $1.49 \pm 0.41 \text{ h}$ , i.e., a value 150 times lower than the physiological MRT.

#### DISCUSSION

This study is the first to report disposition parameters for vitamin C in the calf, a vitamin having a wide range of physiological and biochemical functions. Because AA is not synthesized during the first week of a calf's life (26) it is important to determine the AA requirements in the calf by evaluating the overall rate of AA elimination from the body. Total clearance is the only physiological parameter that measures the overall rate of loss of any endogenous or exogenous product. To estimate the amount of AA that is eliminated daily, it is appropriate to calculate the product of AA plasma

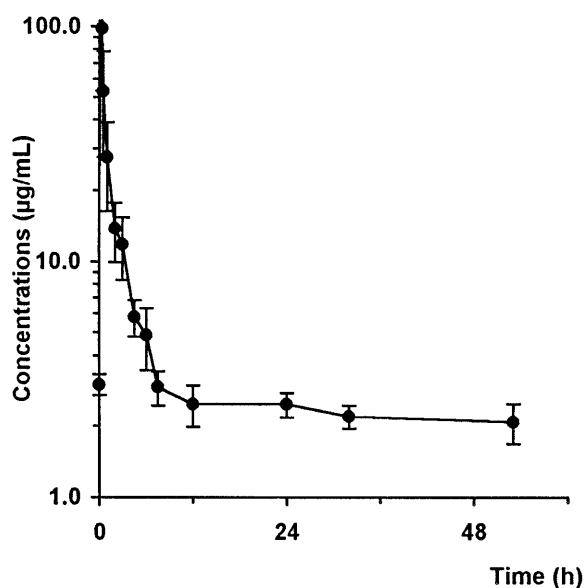


Fig. 9. Semilogarithmic plot of observed (mean  $\pm$  SD) plasma ascorbic acid concentrations ( $\mu\text{g/ml}$ ) vs. time (h) after an intravenous administration of ascorbic acid (3 g) to 5 calves.

clearance and steady-state AA plasma concentration (or the product of AA blood clearance and AA blood concentration). However, when the physiological interpretation is based on clearance, the appropriate fluid (plasma vs. blood) must be selected and the total blood concentration of the analyte of interest must be considered because plasma clearance is only equal to blood clearance where the ratio of blood to plasma concentration is equal to unity (34). In the present experiment, it was shown both in vitro and in vivo that the AA concentrations in blood and plasma were of the same order of magnitude, which suggests that AA does not accumulate in blood cells as observed in other tissues. This is in agreement with results reported earlier that showed that most of the circulating AA occurred in the erythrocytes and plasma and that the average calculated intracellular concentration was the same as the mean plasma concentration (13). In our in vivo experiment, the AA blood-to-plasma activity ratio increased slowly to reach a mean value of  $\sim 2.5$  at 10 h post-intravenous injection. This could partly be due to the high concentration of AA in the leukocytes and platelets, which may be up to 90 times the plasma concentration (13). However the contribution of leukocytes and platelets to the total blood concentration is rather small. It was estimated that leukocytes carried only 10% of blood-borne AA in humans and that the plasma was of prime importance in carrying AA to the tissues (13).

In accordance with our in vitro experiment, the initial AA exchange between plasma and blood cells was very rapid and instantaneous equilibrium could be assumed for the blood cell/plasma partition. In addition, our in vivo experiment showed a second and very slow equilibration process that was only completed after a 10-h delay. It is likely that the first partition corresponds only to a binding of AA to the outside of the erythrocyte, whereas the second process involves an intracellular uptake of AA. The rate of the second equilibration process was so slow that it could be assumed that AA removal from the plasma could not lead to significant reequilibration during organ transit (e.g.,  $< 10 \text{ s}$  for the liver). Thus any measured clearance of AA (plasma, blood) only reflects a plasma elimination process and that plasma (not blood) clearance has to be measured and interpreted in terms of plasma flow rate.

When the cardiac output of a calf is considered [i.e.,  $\sim 73 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (31)] and a mean hematocrit of 40%, the plasma clearance of AA ( $0.68 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) as measured using an AA tracer dose represented only 1.55% of the total plasma flow, indicating a poor overall efficiency of AA removal. In contrast, the plasma clearance obtained with a pharmacological AA dose ( $7.5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) represented 17% of the total plasma flow. This indicates that the kinetic of AA in the calf is nonlinear as reported in humans (7) and in other species, including sheep (6) and horses (25). In humans,  $73 \pm 2\%$  of a pharmacological dose of AA was recovered from the urine in 24 h (14). The reabsorption of AA in the renal tubules is a saturable process, the AA being excreted in large amounts when the plasma concentra-

tion exceeds a renal threshold of  $\sim 8\text{--}15\ \mu\text{g/ml}$  in humans (22, 35). In the present experiment, the overall extraction ratio after the intravenous pharmacological dose of AA (17%) was similar to the plasma flow in the kidney ( $\sim 20\%$  of cardiac plasma output), suggesting that most of the AA was eliminated by a first-pass effect through the kidney when the AA plasma concentration was above a critical value. In contrast, when the physiological AA plasma concentrations were below a critical value, the apparent renal clearance (i.e., irreversible elimination of AA and/or its labeled metabolite) by the kidney represented only 11–13% of the total plasma clearance. This suggests that other major physiological processes of AA elimination exist in the calf. In the rat, urinary excretion accounts for  $\sim 15\%$  of the AA synthesized daily under normal physiological conditions (9). In the guinea pig, only 8% of the label was recovered in urine, demonstrating that under physiological conditions the excretion of AA and its metabolites in the urine is not an important route of elimination (8).

The estimated average rate of AA appearance in the plasma was  $3.4 \pm 1.2\ \text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  in the calf. This amount can be considered as the minimum daily AA requirement either from de novo synthesis or the diet to maintain a mean plasma concentration of 3–4  $\mu\text{g/ml}$ . The rate of AA appearance in the calf was close to that reported in guinea pigs [ $7\ \text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  (16)], but lower than in rat [ $26\ \text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  (9)]. If the calf is unable to synthesize AA, the milk must supply a bioavailable amount of AA of  $\sim 3.4\ \text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ . Although the bioavailability of AA administered orally was not directly measured in the present experiments it should be between that obtained after an intraruminal administration ( $F = 62\%$ ) and after an intraperitoneal administration ( $F = 89\%$ ). Milk bypasses the rumen during suckling, thus avoiding local destruction of AA so that  $<100\%$  bioavailability is due to a lack of AA absorption by the gut and/or first-pass destruction in the liver. This hepatic first effect is probably very low, as demonstrated by the bioavailability obtained after the intraperitoneal administration (the entire dose is drained by the portal vein system after an intraperitoneal administration and undergoes a hepatic first-pass effect). Finally, on the assumption of oral bioavailability of  $\sim 80\%$  and given the AA concentration in milk (8 mg/l) and the daily milk ration (5 l/day), it can be concluded that diet alone cannot provide  $>1\ \text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of AA to a calf. The difference between the AA entry rate and the amount supplied by the diet (i.e.,  $\sim 2.5\ \text{mg/kg}$ ) can only be covered by de novo synthesis or by a depletion of the inborn tissue storage. In the present experiment, the AA body pool was not measured in the newborn calf but was estimated 7 days after parturition to be  $23 \pm 6.8\ \text{mg/kg}$ . This figure is very similar to that reported in humans (1). Consequently, the AA stored in the body of the 7-day-old calf would not be able to supply the daily AA requirement not covered by the milk supply for  $>8\text{--}10$  days. Such a situation is physiologically unlikely and it is reasonable to suggest that the calf is able to synthesize AA at an

early stage. This is at variance with the suggestion that calves are unable to synthesize the vitamin until 2–3 wk after birth (26). It should be noted that our estimates of AA synthesis required to maintain a plasma concentration of 3–4  $\mu\text{g/ml}$  (i.e.,  $\sim 2.5\ \text{mg/kg}$ ) are much lower than the rates of synthesis obtained in other mammals in in vitro liver studies (between 40 and 275  $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) (11). The capacity of calves to synthesize AA could be proved by demonstrating the presence of L-gulanolactone oxidase in the first day of life. This enzyme is missing from all vitamin C-dependent species and is responsible for the conversion of gulonic acid to gulactone in AA synthesis (18).

Due to the limited supply of AA in milk and the impossibility of providing a calf with an exogenous loading dose of AA because of the low kidney elimination threshold (as already discussed), the presence of tissue reserve appeared to be a prerequisite for the calf's well-being. We measured the body pool using both a kinetic approach and a direct measurement of tissue AA content. The total body pool estimated by the kinetic approach at 7 days was 23 mg/kg, as discussed above. The body pool estimated using the tissue concentrations measured was 19 mg/kg. This suggests that the amount of AA in the tissues that was not measured, i.e., skin, digestive tract, bone, and brain, were rather low. However, estimates from the activity data indicated that the unmeasured AA fractions were greater so that no definitive conclusion can be drawn from the present experiment on the contribution of skin, bone, brain, and digestive tract to the AA body pool. Three of the tissues in which AA was measured accounted for most of the total AA body pools: the liver (33%), lung (19%), and muscle (40%). The other tissues measured (spleen, kidney, adipose, endocrine gland) contributed only 8%. In the guinea pig, the largest contribution was also made by the muscle and bone (42% of the total), followed by the liver (23%) and skin (17.7%) (2). The skin might therefore be the tissue not measured in the present experiment that would explain the differences between body pool size estimated from plasma kinetics (23 mg/kg) and that estimated from the direct measurement of tissue AA concentrations (19 mg/kg).

All tissues measured had much higher concentrations of AA than the plasma (from  $8.7\times$  more in adipose to  $473\times$  in adrenal), thus supporting the view that AA uptake by tissues was mostly energy dependent. Similarly, tissue concentrations of DHA were much higher than the plasma DHA concentration ( $23\times$  higher in adipose to  $178\times$  in spleen). In addition, the DHA-to-AA ratio was generally higher in tissues than in plasma, with the notable exception of the adrenal gland (see Table 2). This large tissue accumulation of both AA and DHA requires some specific mechanism. The mechanisms of AA transport and storage in the various tissues are largely unknown. AA is an acid ( $\text{pK}_a = 4.17$ ) that readily undergoes reversible oxidation reduction to DHA. AA is nearly all ionized at physiological pH, whereas DHA is nonionized (24). On this basis, DHA would be expected to more readily cross the cell membrane than AA. This view was supported by others (27)

who also showed that the kidney plays a role in the distribution of vitamin C by oxidizing it to the unionized, membrane-permeable form. On the other hand, it was suggested that DHA has to be reduced to AA before uptake by tissue and possibly into the blood cells (19). Our experiments support the concept of an active rather than a passive vitamin C uptake into tissues as the volume of distribution of AA is dramatically reduced when pharmacological doses of AA are administered (see below).

The physiological meaning of high concentrations of AA in endocrine tissues has been extensively discussed by others (27). For some other tissues, such as skin and bone (not measured in the present experiment), a high AA concentration is consistent with the nutritional requirement for vitamin C in the biosynthesis of collagen (29). For other tissues (muscle, liver, lung), relatively high tissue concentrations can be interpreted as being parts of AA body reserves.

Although definitive information on the distribution of AA was obtained by the measurement of tissue AA concentrations, useful information about both the rate and extent of AA distribution can also be derived from our compartmental model of AA disposition. From the decay pattern of plasma radioactivity, we selected a three-compartment model with elimination from the central compartment only. This is the simplest three-compartment model that is identifiable from plasma AA data. It is different from the three-compartment model proposed in humans (22), which includes a supplementary elimination from one of the peripheral compartments that is viewed as a reversible metabolite pool. Distribution of AA from the central compartment (plasma) to peripheral body compartments (tissues) occurs at various rates and to various extents. The rate of distribution of AA between plasma and a tissue can be limited either by perfusion (plasma flow) or permeability (tissue uptake). It is unlikely that AA distribution to the tissues was limited by permeability. Indeed, from our compartmental analysis, we calculated two high intercompartmental distribution clearances (1,900 and 528 ml·kg<sup>-1</sup>·h<sup>-1</sup> for the first and second peripheral compartments, respectively), the sum (2,428 ml·kg<sup>-1</sup>·h<sup>-1</sup>) being approximately equal to the plasma cardiac output in the calf (2,600 ml·kg<sup>-1</sup>·h<sup>-1</sup>) (31). This suggests that tissue perfusion is the limiting physiological factor to the rate of AA distribution. It also suggests that AA was delivered to the periphery at two rates for two groups of tissues, one receiving a high fraction of cardiac output (~70%) and equilibrates very rapidly with the central compartment, and a second group receiving a lower fraction of the cardiac output (~20%) and equilibrating more slowly.

These two groups of tissues can tentatively be identified by considering the rate and extent of AA distribution to the two peripheral compartments simultaneously and by comparing these parameters with the blood flow to different tissues and the amount of AA actually measured in different tissues at the slaughter time. With the use of this approach, the first peripheral compartment is probably represented by the lung. The

lung is very highly perfused and its AA content (3.58 mg/kg) was very similar to the calculated amount of AA in the first peripheral compartment (3.1 mg/kg). The second peripheral compartment is probably made up mainly of liver and muscle. The contribution of these two tissues to the body pool as obtained from the actual AA concentration was ~14 vs. the 19 mg/kg calculated for the amount located in the second peripheral compartment. In addition, the measured distribution clearance for this second peripheral compartment (528 ml·kg<sup>-1</sup>·h<sup>-1</sup>, i.e., ~20% of plasma cardiac output) is intermediate between the plasma flow to muscle (15% of cardiac output) and the plasma flow to liver (25% of cardiac output) as obtained in different species (30).

For the central and the two peripheral compartments, we calculated the MTT, i.e., the average time taken by AA molecules to leave the compartment after first and possibly subsequent entries into that space. MTT is more descriptive of the intrinsic behavior of the AA molecule within a kinetic space than MRT, because MRT depends on interaction between the different kinetic spaces (32, 33). MTT can be regarded as a measure of how rapidly AA would leave the tissues if the arterial concentrations were suddenly dropped to zero. MTT for the central compartment was very short (11 min), indicating that AA is very rapidly distributed to peripheral tissues. For the first peripheral compartment, the MTT of AA was also relatively short (42 min), but it was much longer in the second peripheral compartment (17.5 h). If the first peripheral compartment is mainly lung tissue, a short MTT would indicate the relative permeability rate of the AA molecule, i.e., AA passage through the lung is very rapid. Inasmuch as the amount in the lung (3.5 mg/kg) corresponds approximately to the calf's AA daily requirement, it is tempting to consider the lung as a rapid mobilization pool, but of a low AA capacity when an immediate specific demand exists (e.g., due to stress). In contrast, liver and muscle represent a large but more slowly mobilized AA pool. The MTT in this high-capacity reservoir (17.5 h) is long compared with the other MTT, but is short in comparison with the total MRT (230 h). This indicates that AA, after leaving the deep tissue stores to gain access to the central compartment, rapidly returns to the peripheral reserve. The number of cycles of the AA around the central compartment (still termed the mean residence number) was 66, indicating a high mean number of passages for each AA molecule through that compartment before final elimination. Such a backward and forward motion of AA molecules between plasma and tissue expresses a unique feature of these deep storage tissues. They have both a high capacity to accumulate AA against a high concentration gradient and the ability to release AA rapidly whenever necessary. The ability to mobilize AA rapidly toward the plasma does not produce a systematic wastage of AA, because AA molecules can quickly be returned to the tissue. This explains the observation that the time necessary to have a 50% drop in the total body AA (6.64 days) when the entry rate is zero, is very prolonged.



Because ruminants appear to be prone to AA deficiency, supplementation with AA is in order. In the present experiment, we have shown that a low dose of AA is highly available by all the tested routes (intramuscular, intraruminal, intraperitoneal), indicating the absence of any absorption-limiting step in the young calf such as the destruction by the ruminal microflora in the adult (10) or a first-pass hepatic effect.

However, the nonlinearity of AA disposition (clearance, volume of distribution) imposes a definite limiting step affecting the possibility of loading tissue by exogenous administration of a massive AA dose. Indeed, when the AA plasma concentration exceeds a critical value ( $\sim 8\text{--}15\ \mu\text{g/ml}$  in humans), the AA filtered by the kidney is no longer reabsorbed due to the saturation of the active transport mechanism (see above). This explains why plasma clearance increases dramatically to reach a value close to the kidney effective plasma flow ( $\sim 450\ \text{ml}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ). A very similar clearance value was obtained in sheep ( $448\ \text{ml}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ) that received a 3-g iv dose of AA (6). The second factor impeding the loading of tissues with a pharmacological dose of AA is the nonlinearity of AA distribution. The  $V_{ss}$  in the calf after a pharmacological AA dose was only 0.658 vs. almost 9 l/kg in normal physiological conditions. This is probably due to saturation of an active carrier-mediated transport of plasma AA. The tissue distribution of AA that was active and operating against a concentration gradient probably becomes a passive phenomenon. As the AA is almost totally in its anionic form at physiological pH, AA is unable to accumulate passively inside the cell. The concentration at which cellular transport is saturated is unknown but it was shown for guinea pig intestine that the apparent Michaelis constant value for transport of AA into brush-border membrane vesicles was  $\sim 50\ \mu\text{g/ml}$  (18). Finally, the saturation of both kidney reabsorption and tissue uptake explains why AA molecules reside in the body (as evaluated by MRT)  $\sim 150$  times less time when the plasma concentration exceeds some critical physiological value. From a practical point of view, it can be concluded that administration of a megadose of AA is not useful. This was previously reported in humans (28). The strategy of supplying an appropriate amount of AA, i.e., without extensive wastage, should encourage the development of a pharmaceutical form that delivers AA at a rate that does not exceed the critical plasma concentration. Such a formulation should release an amount of AA equal to the product of the physiological clearance and the desired increase in plasma concentration. It should not make the plasma levels exceed the critical plasma concentrations, e.g., to increase the plasma concentration of a 50 kg body wt calf from 4 to  $8\ \mu\text{g/ml}$ , the pharmaceutical formulation should ideally release about 8 mg/h according to a zero-order process.

### Perspectives

Apart from its different nutritional and therapeutic applications in cattle, the present experiment emphasized the nature of the kinetic process that controls two

apparently contradictory features of vitamin C physiology, namely a high storage capacity to guarantee vitamin C nutritional requirements (over several weeks) and the possibility to rapidly mobilize vitamin C in the face of an urgent situation (within hours). The existence of a high-capacity pool results from an intracellular accumulation of vitamin C against a 100-fold plasma-to-tissue gradient of concentration, indicating a high intracellular affinity for vitamin C. This intracellular trapping does not impede rapid vitamin C mobilization, because vitamin C kinetics are characterized by a high number of cycles around the central compartment, i.e., vitamin C is permanently entering and leaving the tissue compartment. Thus the equilibrium condition is not static, but characterized by an intensive process of permanent exchange between plasma and tissue. The cellular mechanism explaining not only the entrance and accumulation of vitamin C but also the exit of vitamin C from the cells deserves attention

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