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BIOKINETICS OF SYSTEMICALLY DISTRIBUTED CO-60 IN THE RAT: AN EXPERIMENTAL MODEL USEFUL IN EVALUATING MEDICAL COUNTERMEASURES FOR INTERNAL CONTAMINATION

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Abstract

LBERI, a member of the Medical Countermeasures to Radiologic Threats (MCART) consortium funded by NIAID, was tasked to develop biokinetic models for the distribution of radionuclide threats using the most likely routes of incorporation in both small and large animals. In the present paper, the biokinetics of systemically administered soluble cobalt-60 (⁶⁰Co) have been examined. Male and female jugular-vein-catheterized (JVC) F344 rats received intravenous (IV) doses of 11.2 kBq of ⁶⁰CoCl₂. The distribution of the radiocobalt was followed for 28 d, with tissue sampling done at 1 and 4 h, and at 1, 2, 4, 8, 16, and 28 d. Urine and feces were collected daily. Tissues and excreta were analyzed by gamma pulse height analysis. Within 8d, 93% of the cobalt was eliminated from the body primarily through urine. The highest tissue burdens were found in liver, gastrointestinal (GI) tract, and muscle shortly after administration. These tissues cleared quickly so that by the conclusion of the 28-d study, less than 3% of the recovered dose remained in the body. The results are comparable to published literature values for tissue content of ⁶⁰Co and for excretion patterns up to 30 d after injection. These results will provide the data needed to construct a biokinetic model for the unperturbed biokinetics of ⁶⁰Co in rats, which will subsequently be used to evaluate the impact of administered decorporating agents on organ radiation doses. The animal model described in this paper is representative of that used for other routes of radionuclide administration such as inhalation, ingestion and wound contamination that have been studied at LBERI in support of the MCART and NIAID programs.

Keywords

Biokinetics; cobalt; metabolism; radionuclide

INTRODUCTION

As a member of the Medical Countermeasures to Radiologic Threats (MCART) consortium funded by NIAID, LBERI was tasked to develop biokinetic models for the distribution of radionuclide threats using the most likely routes of incorporation in both small and large

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animals. Furthermore, LBERI was charged with using these animal models to evaluate novel medical countermeasures to decorporate these same radionuclides compared to FDA-approved decorporation agents to reduce whole body burden. The present paper is focused on a biokinetic model for incorporation and biodistribution of Cobalt-60 (^{60}Co) in rats using IV administration that serves as a reference point for other routes of radionuclide incorporation. The animal model described in this paper is representative of that used for other routes of radionuclide administration such as inhalation, ingestion and wound contamination that have been studied at LBERI. Except for procedures specific to different radionuclides and/or routes of administration, the animal model using rats is basically the same.

Cobalt is a naturally occurring element commonly found in soils, plants, and mammals. Only a single stable isotope of cobalt exists, cobalt-59; the other commonly occurring isotopes are radioactive. Cobalt-57 (^{57}Co), cobalt-58 (^{58}Co) and ^{60}Co are the most predominant radioisotopes, which are also of commercial importance with common uses such as sterilizing medical equipment, consumer products, food products, and treating cancer patients (DHHS, 2001). Because of the relatively widespread existence of large sources of ^{60}Co and its use at facilities of varying levels of security, the U.S. Department of Homeland Security considers ^{60}Co to be a radionuclide at risk for use in terrorist activities (e.g., DHS 2003).

The most common scenarios for intakes of radionuclides are inhalation of aerosols, contamination of cuts and scrapes while working with these compounds, or eating and drinking contaminated food or water. Risk of exposure for workers in nuclear plants is nontrivial and cases of internal contamination have been reported (Newton and Rundo 1971; Gupton and Brown 1972; Raghavendran et al. 1978; Hegde et al. 1979; Belezny and Osvay 1994; Davis et al. 2007; Vrba et al. 2007).

Cobalt uptake and distribution in the body is not uncommon. Once inside the body, cobalt is essential for the full functionality of Vitamin B₁₂ (cobalamine) and is quickly converted into this chemical form. B₁₂ is ubiquitous in the body and is essential in several cellular metabolic pathways, including DNA synthesis and regulation. Therefore, upon an accidental intake of large amounts of soluble radioactive cobalt (e.g., ^{60}Co), this material may be incorporated into B₁₂ and distributed throughout the body. This increases the potential for radiological damage, so the need to decorporate cobalt from the body becomes a relevant consideration. Therefore, it is important that the distribution and biokinetics of cobalt are understood and that decorporation strategies be developed. Studies have shown that although non-B₁₂-complexed cobalt quickly translocates throughout the body, it doesn't remain in any tissue for appreciable times; by the conclusion of this study, only 2.4% of the recovered dose remained in the body.

Previous studies have looked at the biokinetics of cobalt in different tissues of the body. Barnaby et al. (1968) followed $^{60}\text{CoCl}_2$ in male albino rats after IV, IP, and oral administration. They administered 14.8 kBq in their intravenous (IV) study. For their short-term retention studies following IP administration, they administered 18.5 kBq; for the intermediate study, they administered 37 kBq; and for the long-term study, they administered 185 kBq. No significant differences were noted between IV and intraperitoneal (IP) administration; thus the authors pooled their data. Onkelinx (1976) followed $^{57}\text{CoCl}_2$ in female Wistar rats following IV administration of 4.7 MBq. The rats were administered the ^{57}Co at one of three ages: 35, 60, or 116 d, and these animals were followed for a subsequent period of 11 d. Their data are represented as concentrations in dpm/g wet tissue. Due to the unknown tissue masses, making comparisons to the present study is difficult and a comparison was not made. Bailey et al. (1989) injected $^{57}\text{Co}(\text{NO}_3)_2$ in either F344 or

HMT rats. (The amount of material administered was not given nor was the sex of each animal identified.) Nishimura et al. (1976) administered either $^{60}\text{CoCl}_2$ (18.5 kBq or 37 kBq) or ^{58}Co -cyanocobalamin to adult Wistar rats. Animals were monitored for 21 d. (The sex of the animals was not reported.) Collier et al. (1989) injected 10 μg of $^{57}\text{Co}(\text{NO}_3)_2$ to female HMT rats. Animals were monitored for 21 d. Thomas et al. (1976) injected 18.5 kBq into male rats (strain not identified) by tail vein injection. Animals on this study were monitored for 309 d. Cikrt and Tichy (1981) administered either 177 $\mu\text{g}/\text{kg}$ or 1770 $\mu\text{g}/\text{kg}$ $^{58}\text{CoCl}_2$ to female Wistar rats by tail vein injection. An inverse dose-dependent elimination in urine was shown. Gregus and Klaassen (1986) administered 0.3mg/kg of $^{57}\text{CoCl}_2$ to male Sprague-Dawley rats by IV administration. Urine and feces were collected and analyzed. Tissue analysis was also conducted and is represented as percentage of the recovered dose (RD)/10 g wet tissue weight. Due to the unknown tissue masses, making comparisons with the results of this study is difficult.

The present study outlines the uptake, distribution, and biokinetics of cobalt in rats after IV administration of a soluble $^{60}\text{CoCl}_2$ complex ranging from 1 d to 28 d. Each tissue was analyzed for radioactivity contents after one of eight different sacrifice times. Urine and feces were collected daily. The results are presented in terms of the dose in each sample as a percentage of the total injected dose. The results of this study are the most comprehensive available in the literature considering that mass balance was achieved in general.

Caution should be taken when extrapolating the present data to humans or other large animal species. Bailey et al. (1989) reported that dogs and baboons excreted less cobalt in feces compared to rats indicating the liver is not processing cobalt as efficiently, thus potentially leading to an increasing liver burden in dogs. However, the goal of the present study was not aimed at determining or extrapolating rat data to humans, as the goal of the present study was to determine the unperturbed biokinetics of cobalt in rats in preparation for conducting decorporation studies. The data obtained will be used to construct a biokinetic model of the unperturbed biokinetics in rats. This model can be used to calculate the organ doses received by the animal. With this information in hand a chelating agent can be administered to the rodent in an attempt to minimize the tissue burdens of the rat. The evaluation of efficacy of chelating agents in support of the FDA Animal Rule is the long term goal of this study and of the entire NIAID internal radionuclide program. This program is also conducting these studies in americium and plutonium contaminated animals prior to the administration chelating agents to decorporate the contamination.

MATERIALS AND METHODS

Animal Handling and Exposure

Male and female F344 rats (Charles River Labs, Kingston, NY, 11 weeks, 140.4–190.1 g females; 210.0–262.0 g males) were surgically implanted with a polyethylene jugular vein catheter (JVC, PE50, 0.023" ID). Upon receipt, the animals were quarantined for 7 d in metabolism cages. Catheter patencies were evaluated using sterile technique the day after arrival and every third day until study initiation and cobalt administration. Animal studies were approved by the Lovelace Respiratory Research Institute (LRRI) Institutional Animal Care and Use Committee; conducted in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International; and carried out in compliance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council 1996). Animals were provided water and food (Harlan Teklad, Madison WI) *ad libitum*. The exception was when animals were removed from their metabolism cages for dose administration. A total of 48 animals (24 males and 24 females) were used for the study. Animals were divided into eight different dosing groups by body mass stratification.

Cobalt-60 chloride ($^{60}\text{CoCl}_2$) in 0.1 M HCl was obtained from Eckert and Ziegler Isotope Products (Valencia, CA). The pH was adjusted to 5.83 with NaOH. Prior to administration, the formulation was filtered through 0.02 μm sterile filters (VWR, Randor, PA) and placed into sterile, multidose injection vials. Aliquots were analyzed by gamma pulse height analysis on a Perkin Elmer 2480 Wizard² Gamma Counter. The formulation concentration was determined to be 55.5 kBq mL⁻¹. An injected dose of 11.1 kBq of $^{60}\text{CoCl}_2$ was administered in a volume of 200 μL through the JVC. The catheter was rinsed with 150 μL of saline to ensure complete delivery. The amount of ^{60}Co injected was calculated by collecting “dummy doses,” which are samples that simulated the amounts administered through the JVC catheter. Instead of being injected into the rat, they were deposited directly into a 20-mL collection vial. Immediately following administration, each rat was returned to their metabolism cage for the duration of the study. Urine and feces were collected on a daily basis and cages were rinsed every third day with deionized water to collect dried urine and fecal residues.

No adverse clinical observations were noted for any of the animals in this study. At predetermined times (1 h, 4 h, and 24 h; 2 d, 4 d, 8 d, 16 d, and 28 d), animals were euthanized by administration of Euthasol (1:9 dilution in sterile saline, 3 mL administration) and pneumothorax. Each rat underwent a complete necropsy with the following tissues separated for radioactivity measurements: blood, liver, spleen, kidneys, lungs, muscle sample (both quadriceps), GI tract, gonads, bone sample (both femurs), pelt, eviscerated carcass, and soft tissue remains. Soft tissue remains contained brain, eyes, thymus, pancreas, heart, tongue, and any other small tissues not defined above.

Radiochemical Processing and Analysis

Small samples (blood, liver, spleen, kidneys, lungs, muscle sample, GI tract, gonads, bone sample, soft tissue, urine, and feces) were placed into 20-mL liquid scintillation vials for direct measurement of ^{60}Co . Large samples (eviscerated carcass, pelt, and cage rinses) were placed in a drying oven (95 °C) for a minimum of 18 h. The samples were transferred to a muffle furnace (550 °C) for a ramped 72-h ashing program. The resulting ash was transferred to liquid scintillation vials for measurement.

Samples were analyzed on the gamma counter utilizing a region of interest that encompassed both the 1.17 and 1.33 MeV gamma rays of ^{60}Co . Each sample was counted for 10 min. The electronic data was retained on LLRI servers for storage, management, manipulation, and analysis. Control charts were maintained to ensure instrument functionality. Counting techniques and efficiencies were verified by analysis of quality control spikes and blanks.

Data Analysis

The total amount of activity recovered from each animal was calculated by summing the activities in all individual tissue and the excreta samples. This summed amount of ^{60}Co was then compared with the injected dose (ID) to obtain the recovery of ^{60}Co for each animal. The data presented herein are then expressed as percent recovered dose (%RD). From this point forward, our %RD is the same as our percent injected dose (%ID). Thus, the material balance experimental design allowed for the determination of the total amount of ^{60}Co recovered for each animal.

Cage rinse samples were proportioned to urine and feces according to the ratio of urine: feces on a given day throughout the sampling period. Due to the difficulty in performing quantitative dissections and collections to obtain complete muscle, skeleton, and blood samples, these quantities were derived from radioactivity measurements performed on

quadriceps muscle and carcass. Blood data is represented as a concentration in %ID mL⁻¹. A carcass sample contained all of the skeletal muscle minus the quadriceps and the skeleton minus the femurs. The quadriceps provided a measurement of the concentration of ⁶⁰Co in muscle, which was converted to the concentration of ⁶⁰Co in total muscle by eqn (1):

$$\text{Total Muscle } ^{60}\text{Co (\%ID)} = ^{60}\text{Co concentration in quadriceps (\%ID g}^{-1}\text{)} \times 0.59 \times \text{Whole-body mass (g)},$$

where 0.59 is the muscle fraction of body mass in a rat (Derelanko 2000).

The content of ⁶⁰Co in the skeleton was then calculated by eqn (2):

$$\text{Total Skeleton } ^{60}\text{Co (\%ID)} = [(^{60}\text{Co in carcass}) - (\text{Total Muscle } ^{60}\text{Co})] + ^{60}\text{Co in femurs(2)}$$

Time-dependent whole-body, tissue, and excreta data were fitted using weighted least squares nonlinear regression to obtain predictive mathematical functions for the uptake, retention, and excretion of ⁶⁰Co. Extrapolation of these functions beyond the data may not be predictive of the true biokinetics of ⁶⁰Co.

Weights in the weighed least squares nonlinear regressions were calculated as the reciprocals of variance estimates obtained from fitted relationships between standard deviations and observed mean values for each sex at different points in time (Neter et al. 1996). To assess sex-related differences in organ uptake and retention curves, a sum of squares reduction F-test for regression (Searle 1971) was applied. This is based on reduction in the error sum of squares from a “full” model containing gender-specific parameters, relative to the error sum of squares from a “reduced” model that did not contain gender-specific regression coefficients.

Repeated (correlated) measurements of whole-body retention and excretion in individual animals across time points necessitated a different approach to assessing sex-related differences for these endpoints. A nonlinear retention (or excretion) model was fit to the data across both sexes (with no sex-specific regression parameters), and the residuals (deviations of observations from model predictions) were analyzed to assess evidence of systematic differences between sex-specific mean values at measurement time points. A weighted least squares repeated measures of a two-way analysis of variance (ANOVA) model with terms for sex, time, and the sex × time interaction, which was applied to make this assessment based on the general estimating equations approach with an autoregressive (order 1) correlation matrix for the error terms in the ANOVA model. In conjunction with observed differences between fitted sex-specific predictive functions, the statistical significance (p-value) of the “main” sex effect in the ANOVA was the criterion used in assessing systematic sex-related differences in retention and excretion.

There was one time point (16 d) at which the data from several organs were clearly inconsistent with data from adjacent measurement times at 8 d and 28 d (and associated model fits). The statistical significance of disproportionate influence of data at 16 d on model fits was assessed with a joint F-test of the studentized residuals (Belsley, Kuh, and Welsch 1980).

All calculations and predictive nonlinear model fits were performed using SAS[®] software, version 9.2.

RESULTS AND DISCUSSION

Material Balance

The radioanalytical results from measurements of all tissues, urine, feces, and cage rinse samples were summed to determine the total recovered ^{60}Co for each animal. The recovered fraction was calculated using the average injected dose of 11.2 ± 0.5 kBq, as determined by analysis of the “dummy doses.” The average recovery across all groups was determined to be $90.0 \pm 4.9\%$ of the administered activity. The group recoveries ranged from 83–95%, only a single study group was below 88% recovery and five of the 8 study groups recovered over 90% of the injected material. These recoveries are adequate for a material balance study. All data have been normalized to the fraction of recovered ^{60}Co for each animal, rather than as a fraction of the average injected activity.

Data Fitting

Empirical functions were applied to the respective whole-body-, organ-, and excreta-specific data sets that represent the average temporal behavior of the data. The functions were multi-component, exponential decay functions. For whole-body and excreta data, time points less than 1 d were excluded, and organ data at the 1 h time point was not included in the fitting parameters to simplify the decay functions. The 16-d data were also excluded from the organ data analyses based on the statistical significance of the disproportionate influence of these data on the fitted functions for several organs ($p < 0.01$). The generalized equation for whole body and tissue contents is shown in eqn (3) unless otherwise noted.

$$\text{Sample}_{(t)} = A_1 e^{-a_1 t} + A_2 e^{-a_2 t} + A_3 e^{-a_3 t} + A_4 e^{-a_4 t}. \quad (3)$$

The coefficients obtained from the data are shown in Table 1. The selection of functions was in general not based totally on semi-empirical and theoretical considerations. This analysis approach does not mimic material-balance calculations. The fitted functions can be used for calculating time-integrated activity days in Bq-d (should be Bq-d) or percentage recovered activity days and, together with an appropriate physical model, could be used to calculate radiation doses. It should be noted that extrapolation of the fitted functions beyond the range of the experimental data (28 d) is not warranted as it may result in inappropriate predictions for the time-related retention patterns.

Whole Body and Tissue Distribution of ^{60}Co

Fig. 1 shows that ^{60}Co was quickly excreted from the body with 67.6% being removed with a half-life of 1.9 hr and 34.5% removed with a half-life of 45.0 h. Subsequently, ^{60}Co continued to be eliminated rapidly from the body; by 1 d only 32.5% remained, which decreased to 12.8% by 4 d; 7.1% by 8 d; and only 2.4% by 28 d remained in the body. The rapid clearance from the body is in agreement with Thomas et al. (1976), who found that 90% cleared the body with a 10.8 h half-time ($T_{1/2}$), 7% with $T_{1/2}$ of 5.3 d, and 2% with $T_{1/2}$ of 31.5 d. Barnaby et al. (1968) showed 17.5% of the material remained at 1 d post administration, which decreased to 8% by day 4 and to 1.9% by day 27. Bailey et al. (1989) found that 18% remained in the body at 1 d, 4.2% at 7 d, and 1.9% at 21 d. Nishimura et al. (1976) found 18% of the administered ^{60}Co activity remained in the body 1 d after administration of $^{60}\text{CoCl}_2$ and 2% after 21 d; however, when ^{58}Co -cyanocobalamin was administered, 93% remained in the body at 1 d and 57% remained in the body at 21 d. Collier et al. (1989) showed 14.7% remained in the body at 1 d post administration, 6.0% at 4 d, and 1.6% at 21 d. The current study showed about a twofold increased retention for the 28 d study period compared to the other published values; however, the present study agrees with the rapid elimination of ^{60}Co from the body. This study also showed a statistically

significant ($p < 0.01$) sex-related difference in whole body retention of cobalt, with males retaining the larger fraction. The curves for both males and females are shown in Fig. 1 and their retention functions are shown in Table 1. In males, 35.7 % remained in the body at 1 d, 14.6% at 4 d, and 2.5% at 28 d following administration. In females, 29.1% remained in the body at 1 d, 11.0% at 4 d, and 2.2% at 28 d following administration.

Liver uptake and retention (Fig. 1) show cobalt rapidly distributes to the liver (26.7 % at 1 h), but the maximum tissue burden likely occurred prior to this collection time; 16.4% of ^{60}Co translocated from the liver with a $T_{1/2}$ of 16.6 h. At 1 d post administration, 10.7% remained, which decreased to 3.9% at 4 d; by 28 d, only 0.4% remained. This study also showed a statistically significant ($p < 0.01$) sex-related difference in liver retention of cobalt, with males retaining the larger fraction. The curves for both males and females are shown in Fig. 1 and their retention functions are shown in Table 1. In males, 13.7 % remained in liver at 1 d, 5.6% at 4 d, and 0.5% at 28 d following administration. In females, 7.7% remained at 1 d, 2.2% at 4 d, and 0.4% at 28 d following administration.

It is evident that the short-term retention in liver tissue for this study differs from those of previous reports. Gregus and Klaassen (1986) found only 4.7% ID in liver at 2 h after cobalt administration, which is significantly less than that for this study. Also in agreement with Gregus and Klaassen are the reported results of Cikrt and Tichy (1981), who reported 3.4% ID remained in liver tissue at 1 d following administration of their low dose and the results in Barnaby et al. (1968) reported of 5.4% remaining at 1 d and 3.9% at 2 d. However, the results of the middle and long-term retentions on the current study show a stronger correlation to those of Barnaby et al. (3.9% on day 4, 1.5% on day 9, and 0.6% on day 27) and Collier et al. (1989) with 0.5% ID at 21 d. Nishimura et al. (1976) showed that 26.4% of the 21 d body burden remained in the liver, which when extrapolated is in general agreement with the 18.3% of the 28 d body burden for this study.

Spleen uptake and retention are shown in Fig. 1. Only 0.1% was present at 1 h. However, the tissue content increased to a maximum of 0.3% at the 4 h time point before decreasing to 0.1% ID at 1 d, 0.08% ID at 8 d, and 0.02% ID at 28 d. This study did not show a statistically significant ($p < 0.01$) sex-related difference in spleen retention of cobalt. Uptake and translocation to spleen can be expressed by eqn (4):

$$\text{Spleen}_{(t)} = 0.58 (1 - \exp^{-2.37t}) * 0.21 (1 - \exp^{-0.063t}) + 2.79 \exp^{-4.06t}, \quad (4)$$

where t is in days.

Although spleen data in the literature are sparse, the results from this study agree with those previously reported. Gregus and Klaassen (1986) reported 0.5% ID remaining in spleen tissue 2 h after administration and Barnaby et al. (1968) found 0.07% ID at 1 d, 0.02% ID at 9 d, and 0.01% ID at 27 d.

Kidney uptake and retention (Fig. 1) show 6.4% present at 1h. The burden decreased to 1.4% at 1d, 0.8% at 4 d, 0.7% at 8 d, and 0.2% at the conclusion of the study. These results are in agreement with Barnaby et al. (1968), who found 0.9% ID remained at 1 d, 0.5% ID at 4 d, and 0.1% ID at 27 d and by Cikrt and Tichy (1981), who reported a 1 d burden of 0.7% ID. Collier et al. (1989) found 0.05% at 21 d. Nishimura et al. (1976) showed that 13.1% of the body burden resided in the kidney at 21 d; when extrapolated, this is in agreement with the 9.5% of the body burden remaining at 28 d on the current study. This study showed a statistically significant ($p < 0.01$) sex-related difference in kidney retention of cobalt with males retaining the higher fraction. The curves for both males and females are shown in Fig. 1 and their retention functions are shown in Table 1. In males, 1.6 % remained in kidney at 1

d, 1.0% at 4 d, and 0.3% at 28 d following administration. In females, 1.3% remained at 1 d, 0.6% at 4 d, and 0.2% at 28 d following administration.

Lung uptake and retention are shown in Fig. 2. Lung data displayed a large amount of data scatter. During the first two days, approximately 1% of the average recovered activity was observed at each of the time points. The content decreased to 0.8% at 4 d, 0.2% at 8d, and 0.2% at 28 d. Barnaby et al. (1968) reported 0.1% ID remained at 1 d, 0.03% ID at 4 d, and 0.01% ID at 27 d. The current study appears to show an approximate tenfold increase in lung retention compared to the Barnaby study. This study did not show a statistically significant ($p<0.01$) sex-related difference in lung retention of cobalt.

Gastrointestinal (GI) tract uptake and retention are shown in Fig. 2. GI showed 13.3% at 1 hr, 5.6% at 1 d, 0.9% at 4 d, 0.4% at 8 d, and 0.1% at 28 d. Barnaby et al. (1968) reported 3.5% ID remains at 1 d, 1.1% ID at 4 d, and 0.3% ID at 27 d; Collier et al. (1989) showed 0.1% at 21 d, which compares to the present study. Nishimura et al. (1976) found 2.9% of the body burden resided in the GI tract at 21 d, which if extrapolated compares to our 28 d data showing 4.5% remaining in the GI tract. The differences may reflect a greater amount of cobalt in the liver for this study. If the ^{60}Co measured in the GI tract samples comes from the contents, rather than ^{60}Co bound to GI tract tissue, then a greater biliary excretion into the GI tract would account for the higher GI tissue contents in this study. This study did not show a statistically significant ($p<0.01$) sex-related difference in GIT retention of cobalt.

Muscle uptake and retention are shown in Fig. 2. Muscle displayed a large initial uptake with 10.9% present at 1 h. Muscle levels decreased to 2.0% at 1 d, 2.3% by 4 d, 0.8% ID at 8 d, and 0.5% by 28 d. Muscle showed a statistically significant ($p<0.01$) sex-related difference in retention of cobalt with males retaining a higher fraction. In Fig. 2, female muscle for the 8 d time point was omitted from analysis since it displayed an abnormally high value when compared to the 16 d and 4 d time points. Barnaby et al. (1968) reported 1.1% ID remained in muscle after 1 d, 0.6% ID at 4 d, and 0.2% at 27 d. Collier et al. (1968) found 0.5% ID remaining in the carcass at 21 d and Nishimura et al. (1976) report 1% ID remaining also at 21 d. Assuming the reported carcass tissue was analyzed in a manner similar to the present study, Collier's muscle fraction would have been 0.3% ID and Nishimura's would have been 0.6% ID, which compares to the current study.

Pelt uptake and retention are shown in Fig. 2. The pelt displayed a large initial uptake with 12.9% present at 1 h, which decreased to 2.1% by 1 d, 1.1% by 4 d, and 0.4% by 28 d. Barnaby et al. (1968) reported 1.5% ID remained after 1 d, 0.4% ID at 4 d, and 0.1% ID at 27 d and Collier et al. (1989) showed that 0.5% ID remained in the pelt at 21 d. Our results correlate well with those previously reported. This study did not show a statistically significant ($p<0.01$) sex-related difference in pelt retention of cobalt.

Skeleton uptake and retention are shown in Fig. 3. Skeleton showed a large initial uptake with 10.6% present at 1 h, which decreased to 10.4% by 1 d, 1.2% by 4 d, 0.8% at 8 d, and 0.6% by 28 d. The results of the present study are consistent with Barnaby et al. (1968), who report 2.0% at 1 d, 0.8% at 4 d, and 0.3% at 27 d. Collier et al. (1989) reported 0.5% ID remaining in carcass at 21 d and Nishimura et al. (1976) reported 1.0% remained also at 21 d. Assuming the carcass data was analyzed in a manner similar to the current study, approximately 0.2% ID (Collier et al. 1989) and 0.4% (Nishimura et al. 1976) remained in bone. This study did not show a statistically significant ($p<0.01$) sex-related difference in bone retention of cobalt.

Testes and ovary uptake and retention are shown in Fig. 3. Both tissues showed minimal initial uptake with 0.5% and 0.4% respectively present at 1 h; content decreased to 0.05% by 4 d and 0.01% by 28 d for testes and 0.01% by 4 d and 0.002% at the conclusion of the

study for ovaries. Testes data in the literature were sparse. Based on our assumed interpretation of the data of Hollins and McCullough (1971) and that of Gregus and Klaassen (1986), approximately 0.2% ID remained in testes at 2 hr and 0.4% ID of the activity remaining in the carcass remained in testes at 10 d. No literature values could be found for ^{60}Co in ovaries.

Soft tissue uptake and clearance are shown in Fig. 3. Soft tissue showed an initial uptake of 2.6% at 1 h and the contents decreased to 0.9% by 1 d, 0.3% by 8 d, and 0.1% by 28 d. This study showed a statistical sex-related difference in soft tissue retention of cobalt with males retaining the higher fraction. The curves for both males and females are shown in Fig. 3 and their retention functions are shown in Table 1. In males, 1.2% remained in soft tissue at 1 d, 0.4% at 4 d, and 0.1% at 28 d following administration. In females, 0.6% remained at 1 d, 0.3% at 4 d, and 0.1% at 28 d following administration. Comparisons to previously reported data were not made since no data were pooled as was done in this study.

Blood uptake and retention are shown in Fig. 3. Rapid clearance was observed from blood, with < 0.5% ID measured at 1 h after injection. The amount remaining in the blood continued to decrease monotonically with time, where by 1 d only 0.12% remained and by 28 d the concentration decreased to $1.5\text{E-}3$ %ID. Blood data in the literature are limited. According to Gregus and Klaassen (1986), between 1 and 2% ID remained in blood at 2 h, whereas Cikrt and Tichy (1981) reported that only 1.0% ID remained in plasma at 1 d post administration. The data of the current study are consistent with blood levels in the literature as reported by Cikrt and Tichy (1981). This study did not show a statistically significant ($p<0.01$) sex-related difference in blood retention of cobalt.

Urinary and Fecal Excretion

Urinary excretion is the main pathway of excretion from the body. Cumulative elimination (Fig. 4) shows that 61% ID was excreted at 1 d after administration, 77% ID at 8 d, and 80% ID at 21 d. This study did not show a statistically significant ($p<0.01$) sex-related difference in urinary excretion of cobalt. The short-term and midrange elimination profiles are consistent with the literature values. Literature values for 1-d urinary eliminations range from 48% ID (Cikrt and Tichy, 1981) to 64 % ID (Bailey et al. 1989). Gregus and Klaassen found that 67.7% was recovered in urine at 1 d, 71% by 2 d, and 72.8% by 4 d. After 7 d, 72% ID had cleared in urine, whereupon the concentrations level off as longer term eliminations at 21 d were found to be 74% for HMT rats and 80% for F344 rats (Bailey et al. 1989). Collier et al. (1989) showed that 53.7% was excreted in urine by 1 d, 58.4% by 4 d, 59.8% by 8 d, and 61.5% by 21 d post administration.

Excretion patterns may be influenced by the age and sex of the animals as well as the amount of cobalt administered. Onkelinx (1976) reported that animals dosed with the same level of activity at different ages excreted cobalt in urine at different rates. Rats administered cobalt at 35, 60, or 116 days of age eliminated 66% ID, 74% ID, and 76% ID respectively at 3 d post administration. The animals in the present study were 77 days of age and eliminated 71.1% at 3 d, which correlates reasonably well with Onkelinx's data. Cikrt and Tichy (1981) reported that elimination at 1 d post administration of cobalt was dependent on the mass level of cobalt administered. Administering $177\text{ }\mu\text{g/kg}$ resulted in a 1 d urinary elimination of 74% ID, whereas administering $1770\text{ }\mu\text{g/kg}$ resulted in a 1 d urinary elimination of 48% ID. These differences were not understood as the high-level elimination is decreased by a tenfold mass increase; however, the ^{60}Co content of the tissues reported do not show a dose dependence. The specific activity of their material was not listed, so it is not possible to compare their results with those from this study at this time.

Thomas et al. (1976) reported a urine to feces ratio of 8.4:1 on day 1, 0.6:1 on day 2, and 1.7:1 on day 4, which is similar to the present 9.8:1 (1 d), 2.0:1 (2 d), and 1.4 (4 d).

The fecal excretion of the present study was determined to be 6% at 1 d after administration, 16% at 8 d, and 17% at 21 d. This study did not show a statistically significant ($p < 0.01$) sex-related difference in fecal excretion of cobalt. This profile indicates that less cobalt was excreted in feces in this study compared to the literature. Data for 1-d fecal excretion ranged from 10.7% ID (Gregus and Klaassen 1986) to 18% ID (Bailey et al. 1989). Gregus and Klaassen also found 13.3% was eliminated at 2 d and 15% by 4 d. Onkelix showed at 3 d post administration to 60-day-old animals that 9.2% was eliminated in feces; 77-day-old animals in this study eliminated 12.5% over the same sampling period. At 7 d post administration, cumulative fecal eliminations ranged 24% ID (Bailey et al. 1989), where they appear to level off since longer term eliminations at 21 d are consistent with those at 7 d. Collier et al. (1989) showed that at 1 d, 15.3% was removed in feces, 18.9% was removed by 4 d, 19.6% was removed by 8 d, and 20.3% by 21 d. Thus, based on the variability seen in the published results, it appears that the fecal excretion of ^{60}Co in this study is reasonably consistent with the literature.

Urinary and fecal excretion profiles can be expressed by eqns (5) and (6):

$$\text{Urine}_{(t)} = 60.89 (1 - \exp^{-2.79t}) + 19.27 (1 - \exp^{-0.26t}) \text{ and} \quad (5)$$

$$\text{Feces}_{(t)} = 16.23 (1 - \exp^{-0.44t}). \quad (6)$$

CONCLUSION

The goal of this ^{60}Co model validation study were to 1) support biokinetic model development for the distribution of ^{60}Co ; 2) to demonstrate proficiency in performing all aspects of this type of injection study in rodents, including the injection technique, metabolism collection, necropsy, in-life husbandry and analytical radiochemical measurements; 3) obtain satisfactory material balance; 4) obtain well characterized biokinetic patterns; and 5) compare the results of this study with previously published results in rats. All of these goals were adequately met. The injection procedure using JVC rats allowed for quantitative delivery of the ^{60}Co chloride solution. The metabolism collection procedures used in this and future studies provide adequate separation of urine and feces. The radiochemical techniques used along with gamma counting of the 1.17 and 1.33 MeV gamma rays from ^{60}Co were a suitable analysis technique, although additional sample processing of the carcass was needed to improve the counting efficiency. The material balance of 90.0% was considered satisfactory. The distribution and retention of ^{60}Co in the various tissues and organs analyzed in this study were broadly in agreement with published results, as were the excretion patterns. However, it is acknowledged that there was significant variability in the published results and, in some cases, interpretation of the published data was uncertain.

We have developed a biokinetic model for IV-injected ^{60}Co in rat. The results of a preliminary study utilizing this method indicate good agreement with previously published results, although some discrepancies were noted. These discrepancies likely arose, at least in part, due to our interpretation of the data from the older studies, and possibly because of the use of both sexes of rats in this study. These results will be used to develop a biokinetic model of injected ^{60}Co in rats using the RATDOSE software (Miller et al. 2012, this issue). This software will calculate the organ doses in advance of chelation decorporation studies to

reduce organ and whole body doses. The evaluation of efficacy of chelating agents in support of the FDA Animal Rule is the long term goal of this study and of the entire NIAID internal radionuclide program. This program is also conducting these studies in americium and plutonium contaminated animals prior to the administration chelating agents to decorporate the contamination.

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Fig. 1. Log-Lin Representations of whole body retention, liver content, spleen content, and kidney content in rats injected intravenously with ^{60}Co .

Fig. 2.
Log-Lin Representations of lung content, GI content, muscle content, and pelt content in rats injected intravenously with ^{60}Co .

Fig. 3. Log-Lin Representations of skeletal content, testes and ovaries content, soft tissue content, and total bone content in rats injected intravenously with ^{60}Co .

Fig. 4.
Log-Lin Representations of urinary and fecal excretion from rats injected intravenously with ^{60}Co .

Table 1

Tissue Distribution Coefficients

$\text{Sample}_{(t)} = A_1 e^{-a_1 t} + A_2 e^{-a_2 t} + A_3 e^{-a_3 t} + A_4 e^{-a_4 t}$						
Sample	A ₁	a ₁	A ₂	a ₂	A ₃	a ₃
Whole Body	72.49	10.55	33.18	0.40	7.46	0.043
Whole Body-Males	47.94	1.97	30.02	0.28	6.09	0.031
Whole Body-Females	86.82	14.75	30.60	0.42	6.86	0.043
Blood	9.43	94.18	0.29	1.00	0.0071	0.055
Liver	16.28	1.08	3.86	0.078		
Liver-Males	16.89	0.94	7.87	0.101		
Liver-Females	13.97	1.01	2.32	0.064		
Kidney	2.91	1.46	0.83	0.049		
Kidney-Males	3.27	2.21	1.32	0.057		
Kidney-Females	2.55	1.31	0.68	0.047		
Lung	0.87	1.05	0.07	0.028		
GI Tract	11.03	0.86	0.69	0.066		
Muscle	5.84	1.37	2.84	0.060		
Muscle-Males	6.66	1.54	3.75	0.066		
Muscle-Females	4.91	1.26	2.32	0.059		
Pelt	4.03	1.36	1.15	0.044		
Ovaries	0.17	1.09	0.005	0.040		
Testes	0.45	1.63	0.056	0.10		
Skeleton	3.13	0.84	1.09	0.025		
Soft Tissue	1.77	1.43	0.37	0.046		
Soft Tissue-Males	1.88	0.93	0.50	0.060		
Soft Tissue-Females	1.54	1.43	0.30	0.034		

Tissue equations not fitted to this functional form are shown in the text.