Tissue Turnover of Aluminum and Ga-67: Effect of Iron Status (43796)

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Abstract. **The purposes of this study were to evaluate the effect of nutritional status in regard to iron on aluminum distribution and turnover and to evaluate Ga-67 as a marker for aluminum. Anemic (n = 27) and normal (n = 30) rats were dosed by gavage with 0.8 mmoles of aluminum and 20 μCi Ga-67 in a 0.75 mol/l citrate solution and sacrificed 1, 3, 6, 9, 15, and 21 days later. Anemic rats generally retained more aluminum in their livers but less in tibias and spleens than normal rats. The half-lives of aluminum in liver (56 vs 17 days), muscle (33 vs 16 days), and serum (12 vs 8 days) were significantly greater in anemic than normal rats, respectively. Total body retention of Ga-67 could be described on the basis of a two-compartment model. The turnover of Ga-67 from the first compartment was rapid (half-life = 0.8 and 0.6 days) in anemic and normal rats, respectively, and was similar to the turnover of Ga-67 from the GI tract (half-life = 0.7 and 0.6 days in anemic and normal rats, respectively). The turnover of Ga-67 from the second compartment was also rapid (2.8 vs 4.0 days in anemic and normal rats, respectively). Anemia affected the retention of Ga-67 more than the retention of aluminum; anemic rats retained more Ga-67 in their livers, spleens, kidneys, hearts, and muscles but less in their tibias than normal rats. In general, Ga-67 was not a satisfactory marker for aluminum distribution and turnover in normal and anemic rats.** [P.S.E.B.M. 1994, Vol 207]

The accumulation of aluminum in tissues during renal failure and perhaps some neurological syndromes has been reported many times (1–3). Several mechanisms by which increased aluminum accumulation can occur (i.e., increased absorption [1, 4–7] and decreased urinary excretion [1, 2, 7–9]), have been studied more extensively than others, such as slowed tissue turnover (10–13).

At least one factor (i.e., nutritional status in regard to iron) may affect tissue aluminum accumulation by several different mechanisms. Cannata *et al.* (6) and Brown *et al.* (14) found that rats fed low levels of iron excreted more aluminum in urine and retained more in liver, spleen, and brain than normal rats. Fernández-Menéndez *et al.* (15) hypothesized that the increased tissue retention of aluminum observed in these studies reflected greater intestinal absorption of aluminum because of decreased iron saturation of transferrin or transferrin-like proteins.

However, changes in iron status and hence transferrin saturation could affect aluminum transport and uptake into cells besides intestinal cells. Fatemi *et al.* (16) estimated that 60% of the aluminum in human plasma (at pH 7.4 with a concentration of 5 μM aluminum) was bound to transferrin. McGregor *et al.* (17) have demonstrated that aluminum bound to transferrin was taken up by cells *in vitro* more efficiently than aluminum bound to citrate.

Moreover, Intragumtornchai *et al.* (18) reported that the cycle time for transferrin to complex with receptors and deliver iron to erythron marrow cells was increased in iron deficiency so that transferrin receptors in anemic rats process only 21% as many transferrin molecules/receptors as those in normal rats. We hypothesized that these changes in iron deficiency anemia would not only increase the half-life of iron but also the half-life of aluminum in tissues. Thus, the pri-

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mary purpose of this study was to assess the effect of iron status on the distribution and turnover of aluminum from tissues.

A second purpose of this work was to examine the usefulness of Ga-67 as a marker of aluminum utilization. Several investigators have utilized Ga-67 to study aluminum transport into the brain (19–21) but Allen and Yokel (22) noted that the distribution of aluminum and Ga-67 in sections of the brain differed. Moreover, chemically Ga-67 resembles iron more than aluminum in terms of charge density and ionic radius (1).

Materials and Methods

Male, weanling Sprague-Dawley rats (Harlan Sprague-Dawley, Madison WI) were fed semipurified diets with adequate iron (50.8 μg Fe/g of diet and 10.5 μg Al/g of diet, by analysis; $n = 30$ in normal group) or an iron deficient diet (11.1 μg Fe/g of diet and 10.3 μg Al/g diet, by analysis; $n = 27$ in anemic group) for 45 days. To increase the severity of the anemia, the rats fed the iron deficient diet (anemic group) were bled weekly by heart puncture while they were anesthetized with Metofane (Pitman-Moore, Inc., Mundelein, IL). The amount of blood drawn varied with the size of the rats and never exceeded 1.4% of body volume (1.0 ml of blood was drawn on Day 6, 1.5 ml on Day 13, and 2.0 ml on Day 20, 27, 34, and 41). The rats fed the control diet (normal group) were exposed to the same procedural stress but little iron depletion (i.e., 0.5 ml of blood was drawn each time).

On Day 24, after being fasted overnight, rats were fed by gavage 1 ml of 0.75 mol/l citrate solution (sodium citrate; Mallinckrodt Inc., Paris, NY) with 0.8 mmole aluminum as aluminum lactate (Pfaltz and Baner Inc., Stamford, CT) and 20 μCi Ga-67 as carrier-free gallium chloride in 0.15 mol/l HCl (Dupont New England Nuclear Products, N. Billerica, MA). The rats were refed diets 1 hr after and sacrificed 1, 3, 6, 9, 15, and 21 days after the gavage treatment.

Animals and Diets. Rats were housed individually in stainless steel, wire bottom cages in a room maintained at 23°–24°C with 12:12-hr light:dark cycles. The facilities and protocols were approved by an institutional animal care and use committee.

The semipurified diets (iron adequate and deficient) were similar in formulations to the AIN-76 diet (23) except that lactalbumin (Teklad Test Diet, Madison, WI) was the protein source as we have reported previously (7, 8). Iron citrate was deleted from the mineral mixture used in the iron deficient diet.

Animals were given free access to deionized water. Food consumption was recorded daily. Rats were weighed twice weekly.

Sample Collection and Laboratory Analysis. Rats were anesthetized with CO_2 and killed by exsanguination. Tissues were excised, cleaned of adhering matter, rinsed with deionized water, and weighed and frozen in polypropylene tubes (Sarstedt Inc., Arlington Heights, IL).

Radioactivity Measurement. Radioactivity due to Ga-67 in rats was measured immediately after the gavage and every day thereafter in a small animal whole-body gamma counter (Canberra Industries, Meridian, CT). Radioactivity in gastrointestinal tract was measured in the whole animal body counter. Radioactivity in all of the other tissues was measured in an automatic gamma-well scintillation counter (Gamma Trac 1191; TM Analytic, Elk Grove Village, IL). A standard sample of Ga-67 was counted before each set of samples on both gamma counters to correct for decay and differences in counting efficiency. The values (cpm) were corrected for background and decay and then converted to becquerels. When counts were less than 100 cpm over background, the data were considered to be invalid and deleted from any calculations and statistical analysis.

Chemical Analyses. Tissues and diets were analyzed for aluminum content using an atomic absorption spectrophotometer with a graphite furnace atomizer (Model 170-70 Polarized Zeeman; Hitachi Tokyo, Japan) (7, 8) and for iron using an atomic absorption spectrophotometer (Perkin Elmer Corp., Norwalk, CT) using procedures described previously (24). Bovine liver (SRM #1577a) standard obtained from the National Institute of Standards and Technology (NIST) was analyzed with several batches of experimental samples. Liver standards were determined to contain 78 ± 3 pmole Al/g ($n = 7$) (estimated but not certified value, 74 pmole Al/g), and 3.69 ± 0.13 μmole Fe/g ($n = 11$) (certified value, 3.47 μmole/g).

Statistical Analysis. Normal and anemic rats were compared in regard to weight and iron status by t test. Aluminum and Ga-67 data were analyzed by two-way analysis of variance (iron status X days after gavage treatment) within the SAS general linear model program (25). Tests for least significant differences were used to differentiate among means for variables significantly affected by treatments.

The aluminum and Ga-67 concentrations in tissues were plotted over time on regular and semilog scales. If the loss of aluminum or Ga-67 appeared to be linear, linear regression equations were calculated (25). We tested the null hypothesis that the independent variable (i.e., days after gavage) did not explain aluminum loss. If the null hypothesis was rejected, we calculated the biological half-lives as $-\ln(2)/\text{slope}$ on the semilog scale (26).

The whole body loss of Ga-67 was not linear on a semilog scale and was analyzed using a pharmacokinetic analysis for a two-compartment model (27). A

linear equation was determined to describe the second compartment (Day 6–13). The resulting extrapolated line was subtracted from the original curve to yield a second straight line. Half-lives were calculated for each compartment.

Results

The anemic rats had lower hematocrits and iron concentrations in spleen, liver, heart, kidney, ulna, and muscle than normal rats (Table I). Moreover, the anemia was severe enough to affect food intake of rats; during Days 1–15 normal and anemic rats consumed 11.6 and 10.2 g feed/day, respectively. After Day 16, rats in the normal group were fed the average amount of feed consumed by the anemic rats on the previous day during the rest of the study (12.4 ± 0.2 g/day). The weights of normal and anemic rats did not differ on Day 22 (148 ± 2 g and 143 ± 2 g, respectively) just before the gavage treatment or on the days that they were sacrificed. However, anemic rats had larger spleens and hearts, but smaller kidneys, per 100 g body wt, than normal rats.

Analysis of variance indicated that anemic rats had more aluminum in livers but less in tibias and spleens than normal rats (Fig. 1). However, lsd analyses indicate that these differences were only obvious at certain time points (Days 1, 6, 15, and 21, in livers; Day 3, in tibias; Days 6 and 9, in spleens; Day 3, in kidneys; and Days 1 and 3, in serum).

The concentration of aluminum in all tissues, but heart, decreased with time after the dosage. Although regression equations using regular scales were found that significantly explained aluminum loss from tissues with time, regression equations explained generally more of variance in the data when the aluminum concentrations were plotted on a natural log scale. For example, the r^2 for the regression equations based on the regular scale (not ln scale) of normal and anemic rats, respectively, were for tibia, 0.10 and 0.53; for spleen, 0.46 and 0.31; for kidney, 0.60 and 0.36; for liver, 0.32 and 0.15; and for muscle, 0.45 and 0.23.

Table I. Iron Status and Organ Weights of Normal and Anemic Rats

	Normal (n = 30)	Anemic (n = 27)
Hematocrit (1)[b]	0.50 ± 0.01[a]	0.25 ± 0.01
Tissue iron (μmole/g wet wt)		
Spleen[b]	2.83 ± 0.11	1.68 ± 0.08
Liver[b]	1.76 ± 0.06	0.50 ± 0.02
Heart[b]	1.49 ± 0.03	0.96 ± 0.04
Kidney[b]	0.83 ± 0.04	0.47 ± 0.03
Ulna[b]	0.48 ± 0.01	0.24 ± 0.01
Muscle[b]	0.23 ± 0.05	0.12 ± 0.04
Tissue weights (g/100 g body wt)		
Spleen[b]	0.27 ± 0.01	0.50 ± 0.03
Liver	4.07 ± 0.13	4.04 ± 0.13
Heart[b]	0.40 ± 0.01	0.57 ± 0.01
Kidneys[c]	0.86 ± 0.01	0.81 ± 0.01
Ulnas	0.18 ± 0.01	0.19 ± 0.01

[a] Mean ± SEM.
[b] Normal and anemic rats differ significantly ($P < 0.0001$).
[c] Normal and anemic rats differ significantly ($P < 0.01$).

Table II lists the calculated regression equations (using ln scale), the P values that indicate whether the equation significantly explained the data, and the r^2 values that indicate the percentage of total variation in the data (tissue aluminum concentrations) that can be explained by the regression lines. The equations explained more than 80% of the total variation in kidney aluminum concentrations for normal and anemic rats and more than 50% of the total variation in tibia and serum aluminum concentrations in normal and anemic rats.

Using the statistically valid equations, we calculated the biological half-lives of aluminum in tissues. The half-lives of aluminum were 8 days in sera and kidneys of normal rats but 16 to 24 days in other soft tissues and tibias of normal rats. The slopes of the equations and presumably the biological half-lives of aluminum in liver, muscle, and serum differed significantly between normal and anemic rats. The half-lives of aluminum in liver and muscle were more than twice as long in anemic than as in normal rats.

Ga-67 Metabolism. Absorption of Ga-67 was calculated as the amount of radioactivity remaining in the animal on Day 1 minus the amount of radioactivity in the gut on Day 1. Normal rats were estimated to absorb −1.9 ± 1.9% and anemic rats were estimated to absorb −4.5 ± 1.1%. Some of the Ga-67 remaining in the small intestine mucosal cells on Day 1 might be absorbed eventually so we calculated absorption using body and gut retention of Ga-67 on Day 3 also. Absorption of Ga-67 of normal (0.5 ± 0.1%) and anemic rats (0.6 ± 0.5%) were slightly higher than the previous calculations. Together these data suggest that absorption of Ga-67 was so low that absorption of Ga-67 cannot be accurately estimated by this means.

Total body retention of Ga-67 did not differ between normal and anemic rats one day after dosing or during the subsequent 14 days (Fig. 2). We attempted to describe the loss of radioactivity from the whole animal by the lines generated in a two-compartment model with distributive (Phase 1) and elimination (Phase 2) phases. The equations expressed as ln radioactivity in body (Y) versus time (x) for Phase 1 are: Normal $Y = 7.03 - 1.096x$, $r^2 = 0.97$; Anemic $Y = 6.68 - 0.889x$, $r^2 = 0.99$; and for Phase 2 are: Normal $Y = 2.88 - 0.172x$, $r^2 = 0.86$; Anemic $Y = 3.89 - 0.250x$, $r^2 = 0.97$. Although this use of a two-

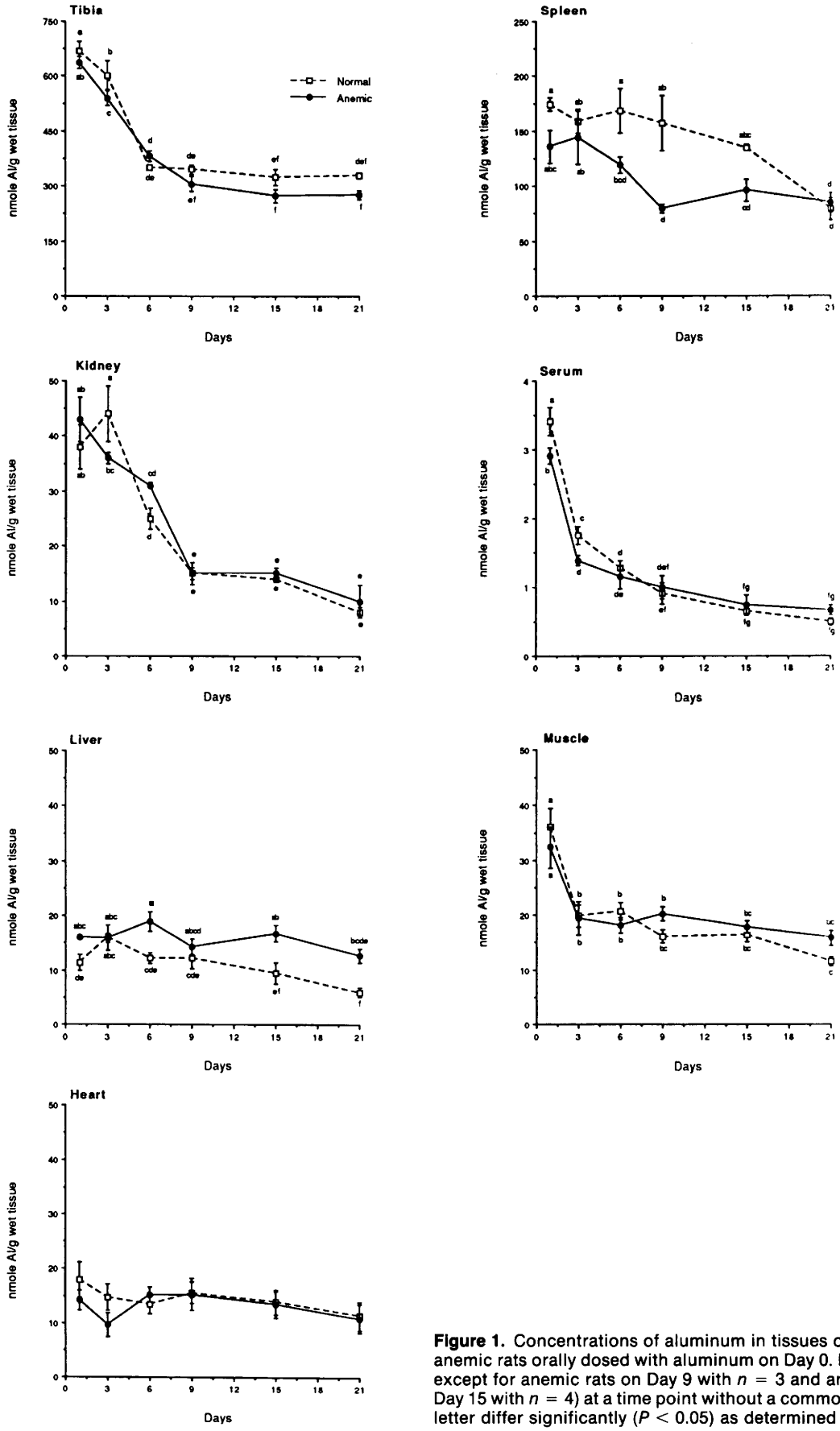

Figure 1. Concentrations of aluminum in tissues of normal and anemic rats orally dosed with aluminum on Day 0. Means (n = 5 except for anemic rats on Day 9 with n = 3 and anemic rats on Day 15 with n = 4) at a time point without a common superscript letter differ significantly ($P < 0.05$) as determined by lsd tests.

Table II. Regression Equations Describing Loss of Aluminum (ln Scale) from Tissues of Normal and Anemic Rats After a Single Oral Dose to Rats

Organ (Y)	Day (X)	Normal				Anemic			
		Equation[a]	P value[b]	r^2	$t_{1/2}$ (days)[c]	Equation[a]	P value[b]	r^2	$t_{1/2}$ (days)[c]
Tibia	1 ~ 21	$Y = 6.35 - 0.0362x$	0.0001	0.58	19	$Y = 6.32 - 0.0402x$	0.0001	0.75	17
Spleen[d]	1 ~ 21	$Y = 5.22 - 0.0293x$	0.0001	0.46	24	$Y = 4.88 - 0.0239x$	0.01	0.28	29
Kidney	1 ~ 21	$Y = 3.74 - 0.0814x$	0.0001	0.82	8	$Y = 3.73 - 0.0705x$	0.0001	0.84	10
Serum[e]	1 ~ 21	$Y = 0.91 - 0.0865x$	0.0001	0.78	8	$Y = 0.64 - 0.057x$	0.0001	0.62	12
Liver[e]	1 ~ 21	$Y = 2.69 - 0.0402x$	0.0005	0.38	17	$Y = 2.85 - 0.0125x$	0.05	0.18	56
Muscle[e]	1 ~ 21	$Y = 3.32 - 0.0428x$	0.0001	0.59	16	$Y = 3.16 - 0.0212x$	0.01	0.27	33
Heart	1 ~ 21	$Y = 2.80 - 0.022x$	NS[f]	—	—	$Y = 2.56 - 0.01x$	NS	—	—

[a] Equations are based on ln (nmoles Al/g tissue).
[b] P values indicate whether ln aluminum declined significantly with time.
[c] Biological half-life ($t_{1/2}$) = $-\ln^2$/slope.
[d] Intercepts of equations describing normal and anemic rats differ significantly ($P < 0.005$).
[e] Slopes of equations describing normal and anemic rats differ significantly ($P < 0.05$).
[f] NS = Not significant.

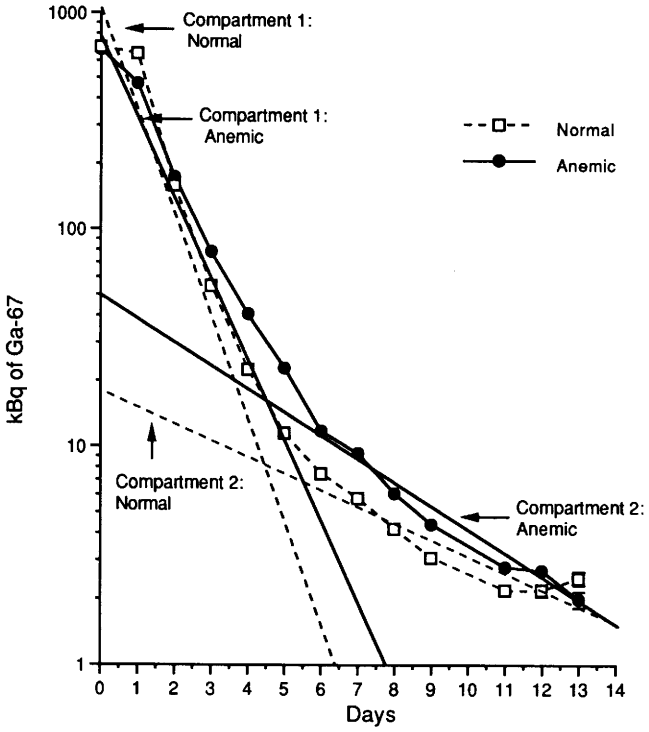

Figure 2. Whole body retention of Ga-67 by normal and anemic rats dosed with aluminum and Ga-67 on Day 0. Pharmacokinetic model reduced the data to two compartments that could be described by linear equations for both anemic and normal rats.

compartment model is somewhat untraditional (27), it is reasonable. The r^2 values suggest that the lines describe the data. Moreover, we think we have identified physiological systems consistent with the two compartments.

Ga-67 in the first compartment had half-lives of 0.6 and 0.8 days in normal and anemic rats, respectively. The radioactivity in the total GI tract was also plotted on a ln scale versus time. The resulting equations (Normal: $Y = 7.17 - 1.10x$, $P < 0.0001$, $r^2 = 0.94$; Anemic: $Y = 6.91 - 0.9x$, $P < 0.0001$, $r^2 = 0.86$) estimated the half-lives of Ga-67 in the GI tracts of normal and anemic rats to be 0.6 and 0.7 days, respectively. Thus elimination of unabsorbed Ga-67 from the GI tract appears to be the logical first phase in the elimination of Ga-67 from the whole body model.

We hypothesized that the second phase in the whole body model reflects the elimination through urine and bile of Ga-67 that was absorbed by the gut mucosa. The estimated half-lives of Ga-67 in Compartment 2 in anemic and normal rats are 2.8 and 4.0 days, respectively. Because Compartment 2 is a crude approximation of the total body after absorption, the elimination of Ga-67 from individual tissues is more interesting. According to analysis of variance, anemic rats retained more Ga-67 in their spleens, kidneys, livers, hearts, and muscles but less Ga-67 in their tibias than normal rats (Fig. 3). The concentration of Ga-67 in all tissues decreased with time after dosage.

The patterns of loss of Ga-67 from tissues appeared different than the patterns of loss of aluminum. We could only define statistically significant linear regression equations to explain the loss of Ga-67 with time from liver and kidney in normal rats and from liver, spleen, tibia, and heart in anemic rats. The equations although statistically significant generally did not explain more than 39% of the variance in the data; the exception was tibias in anemic rats, with a $r^2 = 0.64$. Accordingly, we plotted the Ga-67 concentrations on a ln scale versus time. The resulting equations, except for tibias in anemic rats, were not statistically significant. Thus, no biological half-lives would be calculated.

Discussion

Although much has been published on aluminum toxicity, the metabolism of aluminum, especially orally administered aluminum, is poorly understood partially because analyses are difficult and there have been no aluminum isotopes usable in kinetic studies (1, 28). Moreover, when rats are fed high levels of alumi-

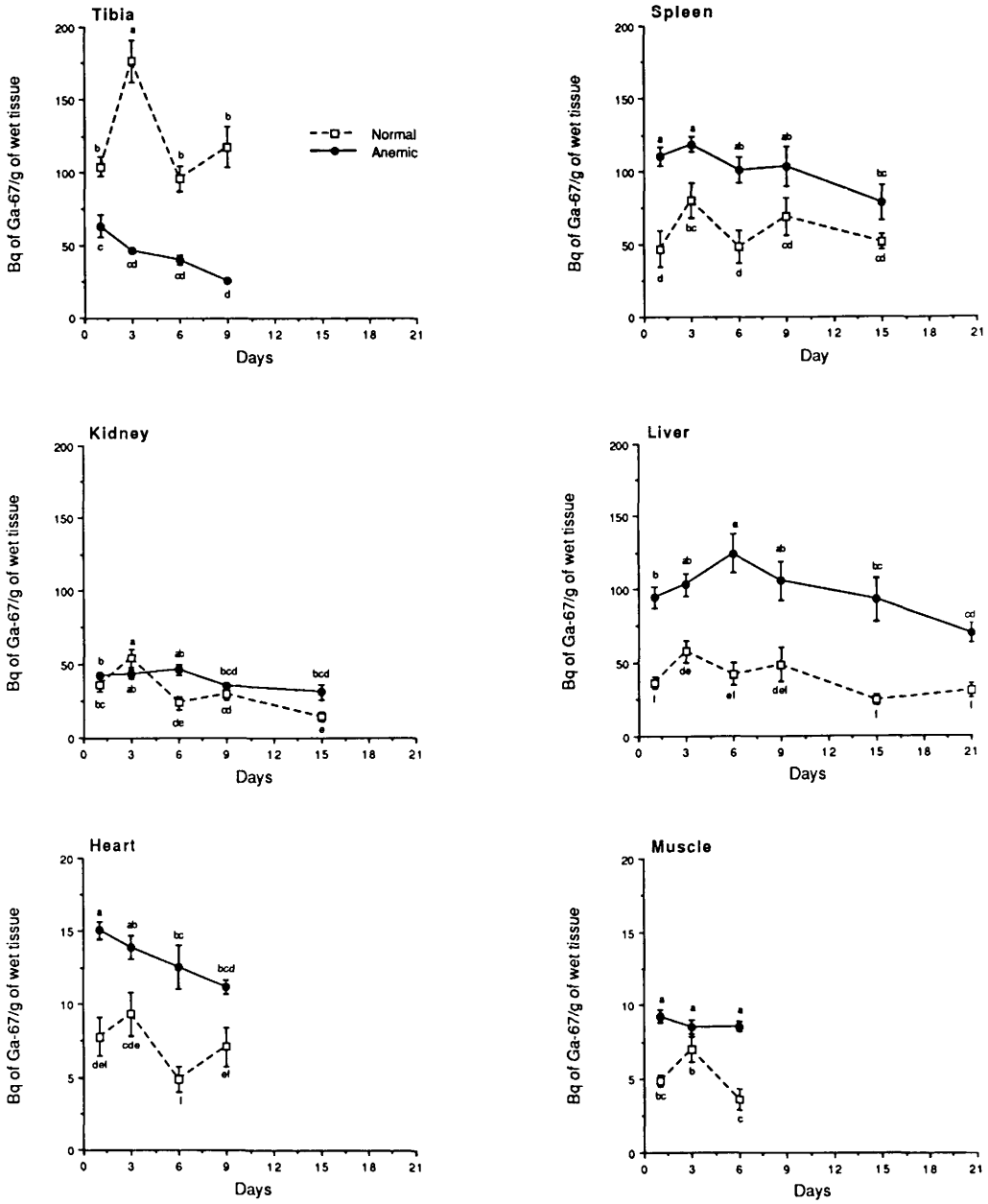

Figure 3. Concentrations of Ga-67 in tissues of normal and anemic rats orally dosed with aluminum and Ga-67 on Day 0. Means (n = 5 except for anemic rats on Day 9 with n = 3 and anemic rats on Day 15 with n = 4) at each time point without a common superscript letter differ significantly ($P < 0.05$) as determined by lsd tests.

num they generally reduce their feed intake (7, 29). In this study, we demonstrated a way to orally administer uniform quantities of aluminum to rats in a manner that allowed us to study tissue turnover of the aluminum.

The gavage administration of a single (0.8 mmole) dose of aluminum in a citrate solution to rats resulted in more retention of aluminum in tissues than feeding moderate amounts (≈40 μmole Al/g) of aluminum as part of a semipurified diet for 28 or 30 days (7, 8). However, the total amount given by gavage was about 18-fold less than the amount consumed in diet. The high retention of aluminum from the gavage dose may reflect several factors including the presence of citrate which enhances aluminum absorption (4, 8), the lack of potential inhibiting factors in the gavage solution that were present in diets (i.e., calcium and iron [8]) and the use of the aluminum lactate which is more soluble than aluminum hydroxide.

Effect of Iron Status on Aluminum Accumulation and Turnover. Our observation on Day 1 that anemic animals had significantly more aluminum in their livers than normal rats is consistent with previous observations (6, 14). During iron deficiency the saturation of iron binding sites on circulating transferrin is reduced and the concentration of circulating transfer-

rin is increased (31). Even though transferrin binds to aluminum much more weakly than to iron (32), it is logical to expect that transport of aluminum to tissues with high concentrations of transferrin receptors (i.e., liver) would be increased in iron deficiency.

The smaller amounts of aluminum in spleen on Day 1 and the shorter half-life of aluminum in sera of anemic than normal rats in this study parallel the observation of Intragumtornchai *et al.* (18) that the rate at which the spleen took up iron-bearing transferrin from plasma was reduced in iron deficiency. As the half-life of aluminum in the spleens of normal and anemic rats did not differ, the continued greater retention of aluminum in spleens of normal than anemic rats appears to reflect primarily differences in the initial uptake of aluminum.

Few investigators have attempted to estimate the biological half-life of orally administered aluminum. Burgess *et al.* (33) calculated the half-life of aluminum in serum of chronic renal patients to be 13 days. We calculated the half-life of aluminum in serum of normal and anemic rats to be 8 and 12 days, respectively. The differences in species and kidney function make comparisons of the data questionable.

Gupta *et al.* (10) estimated the half-life of plasma aluminum in rats given aluminum orally to be 4.9 hr. Lack of agreement between ours and Gupta *et al.'s* estimates probably reflect differences in the dosage of aluminum (148 mg Al/kg vs 8.1 mg Al/kg, respectively), or the salt form administered (aluminum lactate versus aluminum chloride). Pai and Melethil (34) reported that a 10-fold increase in an iv dose of aluminum increased the half-life of aluminum by 66% in rats. It is more likely that differences in the observation period are important. We collected data for 21 days after the oral administration of aluminum; Gupta *et al.* for 10 hr. Wilhem *et al.* (13) observed that estimates of biological half-lives of injected aluminum increased when the observation period increased. They attributed this to incomplete distribution in the short studies.

The potential pathological consequences of differences in aluminum distribution and turnover are not known. The shorter half-life of aluminum in liver of normal rats could reflect increased biliary excretion and reduced toxicity or could reflect more rapid turnover to other tissues. Theoretically, the longer half-lives of aluminum in muscles and livers observed in anemic rats could lead to greater aluminum accumulation and toxicity if aluminum exposure was chronic.

This work demonstrates that aluminum absorption from the gut and the initial transport of aluminum to a tissue are not the only factors affecting aluminum retention in tissues. Tissue turnover can also affect aluminum retention.

Ga-67 as a Marker for Aluminum. Several investigators have suggested that Ga-67 was a good marker for aluminum in whole animals, but these investigators monitored only Ga-67, not both aluminum and Ga-67 utilization (19–21). However, Allen and Yokel (22) reported that injected Ga-67 and aluminum distributed differently in the brain.

We found that anemia increased retention of both aluminum and Ga-67 in livers and reduced the retention of both in tibias. However, the percentage of the dose of aluminum and Ga-67 retained in these tissues differed greatly. On Day 1, anemic rats retained almost 9-fold more of the dose of aluminum than of the dose of Ga-67 in bone (0.08% vs 0.009% of dose/g bone, respectively) and 6-fold less of the dose of aluminum than of the dose of Ga-67 in liver (0.002% vs 0.013% of dose/g liver, respectively). Farrar *et al.* previously noted that gallium, like aluminum, accumulated predominantly in bone of normal rats (30).

Anemia affected the retention of Ga-67 in kidney, muscle, and heart, but had no effect on aluminum retention in these tissues. The greater sensitivity of Ga-67 than aluminum is not surprising. Gallium resembles iron more than aluminum in terms of charge density and ionic radius (1). Martin *et al.* (32) reported that, although human transferrin binds both aluminum and gallium more weakly than iron, transferrin binds gallium more tightly than aluminum by many fold.

The biological half-lives of aluminum and Ga-67 also seemed to differ. The half-life of aluminum in tissues varied from 8 to 56 days, and the loss of aluminum from tissues could be described by a linear plot of the ln aluminum concentration versus time. We could not calculate the biological half-life of Ga-67 in individual tissues on the basis of a linear plot of the ln Ga-67 concentration versus time. Comparison of the turnover of Ga-67 from the second whole body compartment to turnover of aluminum in tissues is questionable. However, elimination of Ga-67 from the second whole body compartment through urine and bile ultimately reflected turnover of Ga-67 from tissues. The half-life of the Ga-67 in the second whole body compartment was shorter than turnover of aluminum in any tissue measured.

Overall, the distribution and turnover of Ga-67 and aluminum were not similar. Ga-67 appeared to be more sensitive to iron status than aluminum. Thus, Ga-67 is not an acceptable marker for aluminum.

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