Effect of hemodialysis on plasma amino acid concentrations in healthy dogs

Denise A. Elliott, BVSc; Stanley L. Marks, BVSc, PhD; Larry D. Cowgill, DVM, PhD; Philip H. Kass, DVM, PhD; Quinton R. Rogers, PhD

Objective—To characterize the effect of maintenance hemodialysis on plasma amino acid concentrations and to quantitate free amino acid losses into the dialysate during hemodialysis in healthy dogs.

Animals—8 healthy adult dogs.

Procedure—Five dogs received hemodialysis treatments 3 times per week for 4 weeks. Plasma amino acid concentrations were evaluated once per week for 4 weeks in each of the 5 dogs prior to hemodialysis (time 0), 90 minutes during hemodialysis, and immediately after hemodialysis (180 minutes). Total free amino acid concentrations and plasma amino acid concentrations (time 0, 90 minutes, and 180 minutes) in the dialysate were evaluated in 3 dogs that received 1 hemodialysis treatment.

Results—Significant time versus week interactions with any plasma amino acid were not detected; however, significant decreases in all plasma amino acid concentrations measured were detected at the midpoint of dialysis (46 ± 2%) and at the end of each dialysis session (38 ± 2%). Mean (± SEM) total free amino acid loss into the dialysate was 2.7 ± 0.2 g or 0.12 g/kg of body weight.

Conclusions and Clinical Relevance—Hemodialysis is associated with significant alterations in plasma amino acid concentrations and loss of free amino acids into the dialysate. Loss of amino acids into the dialysate, coupled with protein calorie malnutrition in uremic patients, may contribute to depletion of amino acid stores.(*Am J Vet Res* 2000;61:869–873)

Alterations in plasma, muscle, and RBC amino acid concentrations have been reported in humans with chronic renal failure receiving either conservative treatment or maintenance hemodialysis.[1-9] Altered plasma amino acid concentrations have also been reported in dogs with experimentally induced or naturally occurring renal failure that has been managed conservatively.[10-12] Abnormalities in the plasma amino acid profile of uremic patients have been attributed to protein calorie malnutrition, deficiency of the excretory and metabolic functions of the diseased kidneys, concurrent endocrine disturbances, and the effect of uremic toxins on the intermediary metabolism of amino acids.[13]

Hemodialysis patients are at a further disadvantage because of the additional contribution of loss of protein and amino acids during dialysis. Total amino acid losses of 6 to 13 g into the dialysate per hemodialysis session have been reported in human patients.[14-20] Furthermore, the hemodialysis procedure per se has adverse catabolic effects on protein metabolism. An enhanced release of amino acids from skeletal muscle has been reported with sham hemodialysis (ie, in vivo passage of blood through a hemodialyzer without circulating dialysate) in clinically normal humans.[21,22] In addition, increased plasma concentrations of 3-methylhistidine in humans in which sham hemodialysis was performed demonstrated the importance of increased protein breakdown in the net catabolic process induced by blood-membrane contact.[22]

To our knowledge, there have been no studies evaluating the effect of maintenance hemodialysis on plasma amino acid concentrations in healthy dogs. The purposes of the study reported here were to characterize plasma amino acid concentrations in healthy dogs receiving maintenance hemodialysis to determine whether changes in amino acid concentrations develop during the hemodialysis procedure, to evaluate the effect of sequential hemodialysis treatments on plasma amino acid concentrations, and to measure the free amino acid losses in dialysate.

Materials and Methods

Dogs—Eight clinically normal 2- to 4-year-old mixed-breed dogs (4 females and 4 males) weighing 18 to 25 kg were obtained from vendors approved by Animal Resources Services of the University of California-Davis and housed in approved facilities on the Davis campus. All animal testing was sanctioned and overseen by campus animal use committees and conformed to all federal, state, and local requirements. All dogs were fed a commercially available adult maintenance diet.[a]

Vascular access—Each dog was sedated with atropine sulfate[b] (0.04 mg/kg of body weight, IM), butorphanol tartrate[c] (0.2 mg/kg, IM), and acepromazine maleate[d] (0.02 mg/kg, IM). Induction and intubation were achieved by administration of thiopental sodium[e] (8 to 10 mg/kg, IV to effect), and anesthesia was maintained with halothane[f] and oxygen. A 40-cm double-lumen transcutaneous silicone hemodialysis catheter[g] was placed in the jugular vein via cutaneous cut down and venotomy and positioned so the tip of the catheter extended into the right atrium. Proper position of the catheter was confirmed by radiography. The incision site was disinfected with povidone iodine ointment[h] and was bandaged to prevent self manipulation. The lumen of each catheter was filled with a 1,000 U/ml heparin sulfate[i] lock, and dogs received aspirin[j] (0.5 mg/kg, PO, q 24 h) to help prevent formation of thromboemboli.

Hemodialysis procedures—Physical examination findings, body weights, and rectal temperatures were recorded for each dog prior to each hemodialysis session. Dialysis was performed with a dialysis delivery system[k] and high-flux dia-

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From the Departments of Molecular Biosciences (Elliott, Rogers), Medicine and Epidemiology (Marks, Cowgill), and Population Health and Reproduction (Kass), School of Veterinary Medicine, University of California, Davis, CA 95616.

lyzers[l] (polysulfone membrane). Prior to each dialysis session, the extracorporeal blood path was filled with sterile saline (0.9% NaCl) solution[m] containing 2,000 U/L of heparin sulfate[i] and recirculated at 200 ml/min for 15 minutes with 2 L/h ultrafiltration. Following testing for sterilant residue, the blood compartment was refreshed with 500 ml of sterile saline solution.

Dialysis was performed on 5 dogs 3 times per week for 4 weeks, using dialyzers reprocessed with sterilant.[n] The hemodialysis directive was: blood flow, 300 ml/min; dialysate flow, 500 ml/min; duration, 3 hours; anticoagulation, heparin sulfate[i] at 100 U/kg prime, 700 to 1,200 U/h adjusted to maintain the activated clotting time between 150 and 200 seconds. The standard composition of dialysate[o] used in this study was: sodium, 145 mmol/L; potassium, 3.0 mmol/L; bicarbonate, 30 mmol/L; chloride, 99 to 112 mmol/L; calcium, 3.0 mmol/L; magnesium, 1.0 mmol/L; and dextrose, 200 mg/dl.

Total dialysate collection—Three dogs received 1 hemodialysis treatment, during which spent dialysate was collected into a 500-gal holding tank. A proportioning pump[p] continuously transferred dialysate as it entered the holding tank into a 5 L jug at a rate of 2.5 ml/min. The holding jug was packed in ice to reduce bacterial growth. At the completion of the hemodialysis session, the total volume of spent dialysate in the holding tank and jug was determined by weight. A thoroughly mixed aliquot was obtained from the holding jug and frozen at –80 C until analyzed for amino acids. The total amino acids in the spent dialysate was calculated as: spent dialysate amino acid concentration (µmol/L) × spent dialysate volume (L).

Sample collection—All dogs had food withheld for 12 hours prior to each hemodialysis session. Venous blood samples were obtained from the hemodialysis catheter for CBC and serum biochemical analyses (cholesterol, CO_2, triglyceride, alkaline phosphatase, magnesium, creatinine, BUN, glucose, phosphate, calcium, total protein, albumin, sodium, potassium, and chloride concentrations) prior to the first hemodialysis session of each week for 4 weeks. Blood samples were also collected for amino acid determination from each dog prior to hemodialysis (time 0), at the midpoint of the hemodialysis session (90 minutes), and immediately after hemodialysis (180 minutes), at the first hemodialysis treatment of each week for 4 weeks. Heparinized samples were placed on ice and centrifuged immediately. Plasma was obtained and deproteinized by addition of an equal volume of 6% sulfosalicylic acid solution. The precipitate was removed via centrifugation, and the supernatant was stored at –80 C until analyzed.

Analytic methods—Complete blood counts were performed by use of an automated cell counter.[q] Serum biochemical analyses were performed by use of an automated multichannel analyzer.[r] Amino acid concentrations were determined by use of an automated analyzer involving cation-exchange chromatography and spectroscopic determination of ninhydrin-derivitized acids.[s]

Statistical analyses—Repeated-measures ANOVA was performed to assess the effect of sampling time (0, 90, and 180 minutes) across weeks, the effect of week independent of sampling time, and any interaction between sampling time and week. If a significant effect was found, orthogonal decomposition of the components was performed. An ANOVA and linear regression analysis were used to assess plasma amino acid and free amino acid concentrations in the dialysate. Values of $P < 0.005$ were considered significant. Results are expressed as mean ± SEM, unless otherwise stated.

Results

There were no significant changes in body weight, CBC, or serum biochemical analyses during the 4-week period (data not shown). Rectal temperature of all dogs remained within reference range limits, and no clinical signs of infection or disease were detected during the experimental period.

Significant differences were not detected between individual plasma amino acid concentrations from week 1 through week 4 at time 0 (data not shown). There were no significant time versus week interactions among plasma amino acids; hence, means were calculated for the amino acid data for each of the 4 weeks at each of the 3 times: before dialysis (time 0), at the midpoint of dialysis (time 90 minutes), and immediately after dialysis (time 180 minutes). Significant decreases in all individual plasma amino acid concentrations were detected during the dialysis period (**Table 1**). The decrease in amino acid concentrations was linear across time with the exception of asparagine, which was nonlinear (quadratic). Most of the decrement in plasma amino acid concentrations occurred by the midpoint of dialysis; that is, plasma amino acid concentrations significantly decreased by 46 ± 2% at the midpoint of dialysis and by 38 ± 2% at the end of the hemodialysis session. The magnitude of the decrease in individual plasma amino acid concentrations during hemodialysis (concentration before dialysis – concentration after dialysis; mmol/L) was correlated with the initial (predialysis) plasma values ($y = 0.355x + 4.18$; $r^2 = 0.93$; $P < 0.001$; **Fig 1**). A similar relationship was also observed when the decrease in individual plasma amino acids at the midpoint of dialysis (predialysis – mid-dialysis concentrations) was compared with the initial (predialysis) values ($y = 0.412x + 5.72$; $r^2 = 0.96$; $P < 0.001$).

Concentrations of free amino acids in the dialysate and their respective plasma amino acid concentrations were tabulated (**Table 2**). Total loss of free amino acids

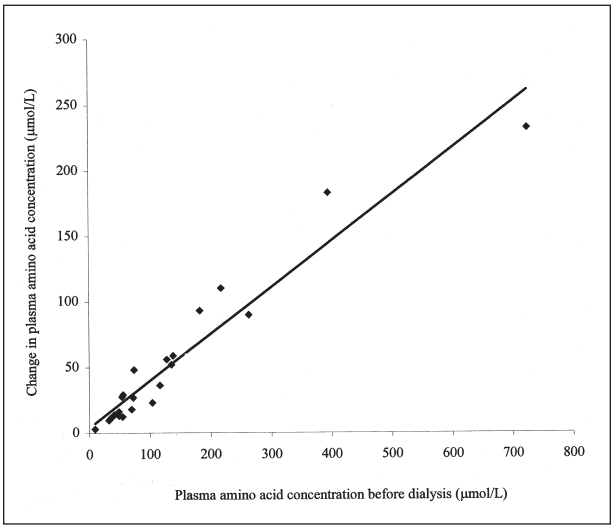

Figure 1—Linear regression model ($y = 0.3548x + 4.1832$) of total change in plasma amino acid concentration (concentration before dialysis – concentration after dialysis) versus initial plasma amino acid concentration before hemodialysis for 5 healthy dogs.

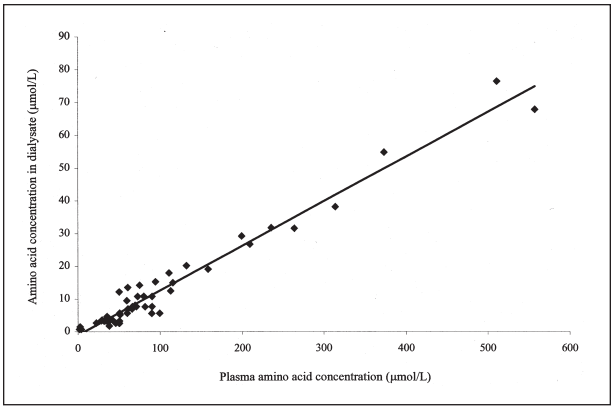

Figure 2—Linear regression model (y = 0.1365x – 1.0709) of free amino acids in dialysate versus mean individual plasma amino acid concentration (mean concentration of each amino acid before, during, and after hemodialysis). Each data point represents a value for 1 amino acid in a single study from 3 healthy dogs.

Table 1—Mean (± SE) plasma amino acid concentrations (μmol/L) before hemodialysis (time 0), at the midpoint of hemodialysis (time 90 minutes), and immediately after hemodialysis (time 180 minutes) in 5 healthy dogs (food was withheld for 12 hours immediately preceding hemodialysis). Hemodialysis was performed 3 times/week for 4 weeks

Plasma amino acid	Before dialysis (time 0)	Mid-dialysis (time 90)	After dialysis (time 180)
Alanine	395 ± 17	186[a] ± 12	212[a] ± 20
Arginine	139 ± 5	74[a] ± 4	84[a] ± 7
Asparagine	32 ± 2	17[a] ± 2	22[a] ± 4
Aspartic acid	9 ± 1	5[a] ± 1	6[a] ± 1
Citrulline	74 ± 4	25[a] ± 3	26[a] ± 2
Glutamine	725 ± 21	446[a] ± 26	493[a] ± 18
Glutamic acid	34 ± 2	23[a] ± 2	23[a] ± 1
Glycine	263 ± 10	141[a] ± 10	173[a] ± 14
Cystine	18 ± 4	7[a] ± 1	5[a] ± 1
Histidine	72 ± 3	42[a] ± 3	45[a] ± 2
Hydroxyproline	56 ± 5	20[a] ± 3	27[a] ± 4
Isoleucine	55 ± 2	32[a] ± 2	42[ab] ± 3
Leucine	104 ± 4	62[a] ± 4	81[ab] ± 6
Lysine	117 ± 6	71[a] ± 5	81[a] ± 7
Methionine	54 ± 18	26[a] ± 2	26[a] ± 1
Ornithine	36 ± 1	24[a] ± 1	24[a] ± 2
Phenylalanine	49 ± 2	29[a] ± 2	26[a] ± 2
Proline	183 ± 11	78[a] ± 7	90[a] ± 11
Serine	139 ± 6	72[a] ± 5	80[a] ± 6
Taurine	49 ± 5	25[a] ± 2	32[ab] ± 3
Threonine	218 ± 7	104[a] ± 6	108[a] ± 7
Tryptophan	70 ± 3	49[a] ± 2	52[a] ± 3
Tyrosine	41 ± 2	23[a] ± 2	27[a] ± 2
Valine	129 ± 4	65[a] ± 4	73[a] ± 7

[a]Significantly ($P < 0.05$) different compared with concentration before dialysis. [b]Significantly ($P < 0.05$) different compared with mid-dialysis concentration.

into the dialysate was 2.7 ± 0.2 g (0.12 g/kg). During a 3-hour hemodialysis session, approximately 5 times the amino acid content of plasma was removed to the dialysate (assuming the typical blood volume of a dog is 0.08 L/kg, and the representative body weight is 22 kg). Clearance of individual amino acids was remarkably similar in magnitude, despite a 3-fold variation in molecular weight. There was a direct correlation between the plasma concentrations of individual amino acids during dialysis and the quantity of free amino acids in dialysate (y = 0.137x – 1.07; $r^2 = 0.96$; $P < 0.001$; **Fig 2**).

Table 2—Mean (± SE) plasma amino acid concentration (μmol/L) and total loss of amino acids to the dialysate before, during, and after hemodialysis in 3 healthy dogs (food was withheld for 12 hours immediately preceding hemodialysis). Hemodialysis was performed once in each dog

Plasma amino acid	Before dialysis (time 0)	Mid-dialysis (time 90 minutes)	After dialysis (time 180 minutes)	Total dialysate (mg)
Alanine	406 ± 67	213[a] ± 9	193[a] ± 16	296 ± 46
Arginine	114 ± 16	76 ± 11	60[a] ± 12	96 ± 9
Asparagine	39 ± 6	19 ± 4	16[a] ± 3	N/D
Aspartic acid	3 ± 1	3 ± 1	3 ± 1	14 ± 5
Citrulline	31 ± 4	17 ± 6	11[a] ± 2	N/D
Cystine	56 ± 5	36[a] ± 3	38[a] ± 7	N/D
Glutamine	601 ± 80	450 ± 41	40 ± 15	958 ± 170
Glutamic acid	46 ± 1	391[a] ± 47	24[a] ± 4	N/D
Glycine	246 ± 29	161 ± 25	134[a] ± 20	182 ± 5
Histidine	66 ± 4	47 ± 7	37[a] ± 9	77 ± 11
Hydroxyproline	30 ± 9	13 ± 2	13 ± 3	N/D
Isoleucine	53 ± 6	25[a] ± 1	27[a] ± 4	45 ± 3
Leucine	105 ± 6	56[a] ± 6	56[a] ± 6	96 ± 4
Lysine	150 ± 25	88 ± 6	70[a] ± 4	185 ± 34
Methionine	43 ± 5	25 ± 5	20[a] ± 3	47 ± 3
Ornithine	16 ± 3	11 ± 2	6[a] ± 1	N/D
Phenylalanine	50 ± 8	33 ± 5	35 ± 5	53 ± 5
Proline	93 ± 16	58 ± 10	34[a] ± 3	148 ± 6
Serine	125 ± 8	81 ± 15	68[a] ± 10	132 ± 17
Taurine	54 ± 7	39 ± 4	32[a] ± 4	47 ± 11
Threonine	149 ± 42	92 ± 28	71 ± 19	165 ± 23
Tryptophan	77 ± 4	69 ± 9	40 ± 16	N/D
Tyrosine	37 ± 5	20[a] ± 3	20[a] ± 3	46 ± 4
Valine	116 ± 10	62[a] ± 7	50[a] ± 7	88 ± 8

[a]Significantly ($P < 0.05$) different compared with predialysis concentration. N/D = Not detectable.

Discussion

Results of the present study indicated that there is a negative amino acid balance during dialysis, because concentrations of all amino acids were significantly reduced during the period of dialysis with consistent and significant losses of free amino acids into the dialysate.

The 38% decrease in plasma amino acid concentrations during hemodialysis in the dogs of our study is similar to the 23 to 49% reduction in plasma amino acid concentrations reported in humans.[16-20] The minor differences in the decrease in plasma amino acid concentrations may reflect methodological differences in fasting versus postprandial sample collection, timing of the postdialysis sample, and efficacy of the hemodialysis directive used. The dogs in this study were all in good general condition with stable body weights and no clinical or laboratory signs of protein malnutrition. Furthermore, metabolic abnormalities such as metabolic acidosis, infective, or inflammatory conditions that would affect protein and amino acid metabolism were not apparent. The amino acid abnormalities that arose during the hemodialytic process could not be attributed to protein calorie malnutrition or nutritional status in general but appeared to be a consequence of loss of amino acids during the dialysis procedure or the catabolic influence of the dialysis procedure per se.

Results of our study indicated that there was a loss of approximately 3 g of free amino acids in dogs weighing approximately 22 kg into the dialysate (0.12 g/kg). These findings are similar to those in humans (weighing approx 70 kg), for which losses of free amino acids into the dialysate have been reported to be 6 to 12 g per dialysis session (0.09 to 0.17

g/kg).[14,16-20] Kopple et al[14] reported 6.3 g of amino acid losses during an 11-hour hemodialysis period with Kiil dialyzers and cuprophane membranes. Wolfson et al[16] described amino acid losses of 8.1 ± 1.1 g with a 5-hour dialysis period, using various low flux dialyzers. Ono et al[17] observed amino acid losses of 6.6 ± 0.5 g with a 5-hour dialysis period, using a cuprammonium rayon hollow fiber dialyzer. Gutierrez et al[18] reported an amino acid removal of 7.9 ± 0.4 g with a cellulose acetate[t] or hemophan dialyzer.[u] Ikizler et al[19] described similar amino acid losses in male patients who ate a small meal and underwent dialysis, using a low flux cuprophan dialyzer (7.2 ± 0.9 g), a low flux polymethacrylate dialyzer (6.1 ± 0.5 g), and a high flux polysulfone dialyzer (8.0 ± 2.8 g). When the polysulfone membrane was reused 6 times, the free amino acid losses increased to 12.2 ± 1.5 g. Hemodialysis performed with high flux cellulose triacetate CT190 dialyzers resulted in losses of total amino acids that averaged 10.0 ± 0.9 g per hemodialysis session.[20] It is noteworthy that the amino acid losses reported in these studies were similar, although various standard dialyzers had been used. Furthermore, several grams of peptides and protein are also lost during hemodialysis treatment.[14,19] Thus, the total free and bound amino acid losses during hemodialysis may be even greater than indicated by this study. It has been suggested that dialysis is a catabolic event caused by increased urea appearance during hemodialysis,[23,24] a negative nitrogen balance on the days in which hemodialysis is performed, regardless of protein intake,[25] or possibly because of free amino acid losses during hemodialysis treatment. Amount of amino acids recovered from the dialysate does not solely account for the observed reduction in plasma amino acid concentrations. Decreases in the plasma amino acid pool account for less than 30% (0.77 g) of the total loss of amino acids into dialysate (with a typical body weight of 22 kg, a volume of distribution of 0.25 L/kg, and a mean amino acid change of 0.12 g/L during dialysis). This discrepancy suggests that 70% of the amino acids lost into the dialysate are obtained from intracellular sites. In clinically normal humans that underwent 150 minutes of sham dialysis (a procedure by which blood is passed through a dialyzer without circulating dialysate), the interaction between blood and the dialyzer membrane elicits an increased net efflux of amino acids from the leg musculature corresponding to approximately 20 g of protein catabolism.[18,22,26] Furthermore, increased plasma concentrations of 3-methylhistidine in sham hemodialysis in normal humans supports the importance of increased protein breakdown in the net catabolic process induced by blood-membrane contact.[22] The observed catabolic process is likely a bioincompatibility reaction, whereby interaction of blood with the hemodialyzer membrane activates complement via the alternative pathway, eliciting the release of interleukin-1 and tumor necrosis factor from macrophages.[27] These cytokines may act synergistically to induce or mediate skeletal muscle catabolism.[28-30] Therefore, in addition to the reduction in plasma amino acid concentrations, there may also be a considerable disruption in intracellular free amino acid pools associated with catabolism of protein and peptides to replace the intracellular amino acid losses.

In patients that are in good clinical condition, moderate losses of amino acids during hemodialysis should easily be compensated for by adequate nutritional intake. Indeed, in our study there were no alterations in baseline plasma amino acid concentrations during the 4-week period. However, many hemodialysis patients suffer from anorexia and gastrointestinal or other disturbances that may grossly impair or preclude oral intake of nutrients. Therefore, the loss of amino acids with each dialysis treatment, coupled with a decreased protein intake in a uremic patient, can lead to a gradual depletion of amino acid stores, particularly if the daily energy intake is inadequate. Oral intake of nutrients immediately before or during hemodialysis may improve nitrogen balance,[17] although gastric emptying may be impaired in some human patients undergoing dialysis.[31] Methods to improve nitrogen balance during the dialysis procedure include oral or IV administration of essential amino acids[16,32,33] or addition of glucose or amino acids to the dialysate.[14,34] These practices are expensive, and the potential benefits of hemodialytic nutrition are not well-defined. Further studies are necessary to determine the benefit of nutritional needs during dialysis.

Our results revealed that hemodialysis decreases plasma amino acid concentrations. The etiopathogenesis of the abnormality is most likely multifactorial but includes amino acid losses into the dialysate, possibly coupled with increased catabolism. The clinical consequences and importance of the increased amino acid losses need to be more clearly defined. Although a total loss of 3 g of free amino acids per dialysis session represents a small fraction (8 to 10%) of the patient's daily protein intake, this may be a loss that uremic patients cannot afford.

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[a]Dealers Pride Original Formula, Ralston Purina Co, St Louis, Mo.
[b]Atropine sulfate, 0.4 mg/ml, Elkins-Sinn Inc, Cherry Hill, NJ.
[c]Torbutrol, 0.5 mg/ml, Fort Dodge Laboratories, Fort Dodge, Iowa.
[d]Acepromazine maleate, 10 mg/ml, Fermenta Animal Health Co, Kansas City, Mo.
[e]Pentothal, 25 mg/ml, Sanofi Animal Health, Overland Park, Kan.
[f]Halothane USP, Fort Dodge Laboratories, Fort Dodge, Iowa.
[g]PermCath7, Quinton Instrument Co, Bothell, Wash.
[h]Betadine Ointment, Vedco, St Joseph, Mo.
[i]Heparin sodium injection, USP, 1,000 U/ml, Elkins-Dinn Inc, Cherry Hill, NJ.
[j]Children's Aspirin, Rugby, Norcross, Ga.
[k]COBE Laboratories, Lakewood, Colo.
[l]Fresenius USA Inc, Walnut Creek, Calif.
[m]Baxter Healthcare Corp, Deerfield, Ill.
[n]Enzodine, Biomedical Development Corp, San Antonio, Tex.
[o]3-K Renalyte[7] Rx Acid Concentrate for Bicarbonate Dialysis, COBE Laboratories Inc, Lakewood, Colo.
[p]Masterflex7, Cole-Parmer Instrument Co, Chicago, Ill.
[q]System 9000 Hematology Series Cell Counter, Baxter Instruments, Deerfield, Ill.
[r]Cobas Mira Plus CC, Roche Diagnostics, Branchburg, NJ.
[s]Model 7300, Beckman Instruments, Palo Alto, Calif.
[t]Baxter CA 170G dialyzer, Baxter Healthcare Corp, Deerfield, Ill.
[u]Gambro GFS Plus 20 dialyzer, Gambro Renal Products, COBE Laboratories Inc, Lakewood, Colo.

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