The Effect of Hemodialysis on Protein Metabolism

A Leucine Kinetic Study

Victoria S. Lim, Dennis M. Bier, Michael J. Flanigan, and Sam T. Sum-Ping

Departments of Internal Medicine and Anesthesia, University of Iowa College of Medicine, Iowa City, Iowa 52242; and Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri 63110

Abstract

To assess the effect of hemodialysis on protein metabolism, leucine flux was measured in seven patients before, during, and after high efficiency hemodialysis using cuprophane dialyzers and bicarbonate dialysate during a primed-constant infusion of L-[1-^{13}C]leucine. The kinetics [μmol/kg per h, mean±SD] are as follows: leucine appearance into the plasma leucine pool was 86±28, 80±28, and 85±25, respectively, before, during, and after dialysis. Leucine appearance into the whole body leucine pool, derived from plasma [1-^{13}C]α-ketoisocaproate enrichment, was 118±31, 118±31, and 114±28 before, during, and after dialysis, respectively. In the absence of leucine intake, appearance rate reflects protein degradation, which was clearly unaffected by dialysis. Leucine oxidation rate was 17.3±7.8 before, decreased to 13.8±7.8 during, and increased to 18.9±10.3 after dialysis ($P = 0.027$). Leucine protein incorporation was 101±26 before, was reduced to 89±23 during, and returned to 95±23 after dialysis ($P = 0.13$). Leucine net balance, the difference between leucine protein incorporation and leucine release from endogenous degradation, was −17.3±7.8 before, decreased to −28.5±11.0 during, and returned to −18.9±10.3 after dialysis ($P < 0.0001$). This markedly more negative leucine balance during dialysis was accountable by dialysate leucine loss, which was 14.4±6.2 μmol/kg per h. These data suggest that hemodialysis using a cuprophane membrane did not acutely induce protein degradation. It was, nevertheless, a net catabolic event because protein synthesis was reduced and amino acid was lost into the dialysate. (*J. Clin. Invest.* 1993 91:2429–2436.) Key words: hemodialysis • leucine kinetics • protein flux

Introduction

It is generally believed that hemodialysis is a protein catabolic process (1–3). This notion stems from the observation of that blood urea nitrogen rebounds rapidly after dialysis and the fact that 6–8 g of amino acids are lost to the dialysate per treatment (4–6). Furthermore, Farrell and colleagues (7) and Ward et al. (8) noted that urea generation rate, calculated from urea kinetics, is 30% higher during dialysis than during the interdialytic period. Using metabolic balance technique, Borah et al. (9) noted that nitrogen balance is always less on the day of dialysis irrespective of the quantity of protein intake, and we have noted that dialysate nitrogen loss per day tends to be greater when the interdialytic interval is shorter (10). None of these studies, however, provide unequivocal evidence of dialysis-induced protein breakdown. The rapid rebound of urea nitrogen after dialysis could be explained on the basis of intercompartmental redistribution and equilibration (11). Nitrogen balance studies are limited by their inherent methodological inaccuracies and are able to measure only net changes in protein metabolism (12). The mathematics of urea kinetics permit calculation of urea generation rate per dialysis cycle (from the end of one dialysis to the end of the next) but cannot reliably sort out the differences between the intra- and the interdialytic periods (13). The most convincing evidence of hemodialysis-induced protein breakdown is reported by Gutierrez and colleagues (14), showing that circulation of blood through a dialyzer without dialysate increased amino acid release from the leg, suggesting that contact of blood with the dialysis membrane led to proteolysis.

To address more directly the issue of dialysis-induced protein catabolism, we used the technique of primed-constant infusion of L-[1-^{13}C]leucine, a tracer amino acid, for estimation of whole body protein flux before, during, and for 4 h after hemodialysis.

Methods

Study subjects and dialysis prescription. Seven hemodialysis patients were recruited for this study; their demographic, nutritional, and dialysis status are listed in Table I. All participants were clinically stable and without intercurrent acute illness. There were four women and three men; their ages ranged from 20 to 56 yr. Diagnoses included four with chronic glomerulonephritis and one each with hypertensive nephrosclerosis, adult polycystic kidney disease, and medullary cystic kidney disease. All patients were in stable clinical condition and none had diabetes mellitus. With the exception of J.D., serum albumin was normal in all participants. Their medication consisted of phosphate binders and multivitamins and, in J.D., Enalapril (Merck, Sharp & Dohme, West Point, PA). No subjects were receiving hormone (nandrolone decanoate, insulin, glucocorticoid, or thyroid replacement) or immunosuppressive agents that could alter protein metabolism. Dialysis consisted of high efficiency hemodialysis using the Gambro single pass delivery system (CGH Medical Inc., Lakewood, CO) with an ultrafiltration controller and bicarbonate dialysate. Dialysis was performed three times a week and 3 h per session using Gambro 6 N cuprophane dialyzers (1.6 m^2). Blood flow rates were 400 ml/min and dialysate flow rates were 500 ml/min. Their Kt/V ranged from 0.71 to 1.77 and their normalized protein catabolic rate (NPCR)[1] ranged from 0.77 to 1.41 g/kg per d. (The values listed for RF underestimated his true

This work presented in abstract form at the American Society of Nephrology Meeting, Washington, DC, 2–5 December 1990.

Address correspondence to V. S. Lim, M.D., Nephrology Division, Department of Internal Medicine, W 336-16, University of Iowa Hospitals, Iowa City, IA 54442.

Received for publication 4 April 1992 and in revised form 11 January 1993.

J. Clin. Invest.

0021-9738/93/06/2429/08 $2.00
Volume 91, June 1993, 2429–2436

1. *Abbreviations used in this paper:* KIC, α-ketoisocaproate; NPCR, normalized protein catabolic rate; PCR, protein catabolic rate.

Table I. Demographic, Nutritional, and Dialysis Status of the Study Subjects

Subject	Age/Sex	BMI	Serum albumin	*Kt/V**	NPCR
		wt/ht²	*g/dl*		*g/kg per d*
I.A.	56/F	29.6	3.9	1.31	0.98
J.D.	32/M	20.5	3.2	0.71	0.77
E.B.	54/F	31.1	4.2	1.36	1.41
T.D.	23/F	17.2	4.1	1.77	1.14
R.F.	46/M	26.8	4.2	0.86	0.97
V.M.	39/F	21.8	4.7	1.11	0.81
R.S.	20/M	19.3	4.7	1.02	0.94

BMI, body mass index; F, female; M, male. *Kt/V* and NPCR represent dialysis adequacy index and estimated daily protein intake (see Methods).
* Urea.

Kt/V and protein catabolic rate (PCR) as he regularly dialyzed at home for 4.5–5 h three times a week.) Both parameters were determined from the 3-h dialysis sessions performed preceding the experiment. *Kt/V* of urea represents the "dose of dialysis" and is used as a dialysis adequacy index, where k equals dialyzer urea clearance (ml/min), t is time on dialysis (min), and V is volume of urea distribution (ml). Dialyzer clearance was measured from the spent dialysate volume and dialysate and blood urea nitrogen concentration. Dialysis time was recorded. Volume of urea distribution was calculated from the single-pool variable volume kinetics (13, 15). Urea appearance rate or urea generation rate (G) was also derived from the urea kinetic equations. PCR = $[(G \times 1.44) + 1.7]/0.154$ (9). NPCR expresses PCR per body weight, which was normalized for a standard urea volume of distribution of 58%, thus NPCR = PCR/(V/0.58). In steady state, NPCR is an estimate of dietary protein intake. Vascular access recirculation was < 7% in all subjects. Study dialyses were performed as described with the exception that the time was shortened to 2.5 h to accommodate the experiment.

Experimental design

Materials. L-[1-^{13}C]leucine, (99 atom% ^{13}C), and NaH^{13}-CO_3, 95% atom% ^{13}C, were purchased from Merck Sharp & Dohme/Isotopes, Dorval, Quebec, Canada. Infusates were prepared aseptically the afternoon before the experiment.

Procedures. Subjects were admitted to the Clinical Research Center the day before the study. After a 12-h overnight fast, leucine infusion was started at 0700 the next morning. Gauge 20 needles were inserted into the arterial and the venous ends of the vascular access, the former for blood sampling and the latter for infusion. Isotopic [1-^{13}C]leucine (see below) was infused for 4 h from 0700 to 1100 hours before dialysis, continued for 2.5 h during high efficiency hemodialysis, and for 4 h after dialysis (total infusion time was 10.5 h). The first 2 h of the infusion was used to achieve isotopic equilibrium; data from the third and fourth hours served as the predialysis baseline to which dialysis and postdialysis values were compared. CO_2 production rate (FCO_2), atom percent excess of [1-^{13}C]leucine and [1-^{13}C]KIC in the plasma, and atom percent excess of $^{13}CO_2$ in the expired gas were quantitated before infusion and every 30 min for 2 h before, during, and 4 h after dialysis. Additionally, total plasma leucine concentration was measured before, during, and after dialysis. Spent dialysate was also collected during the study and, after appropriate mixing, aliquots were taken for measurement of leucine concentration.

The protocol was approved by the Committee on Human Research of the University of Iowa College of Medicine.

Leucine turnover kinetics. Leucine kinetics were measured by a primed-constant infusion technique during substrate and isotopic steady state (16–18). The priming solution consisted of 4.0 μmol/kg of L-[1-^{13}C]leucine and 0.11 mg/kg of NaH$^{13}CO_3$. In anticipation of leucine loss into the dialysate, the constant infusion was given at the rate of 6.0 μmol/kg per h.

Quantitation of amino acid metabolism was on the basis of the relationship stating that $Q = S + C = B + I$, where Q is flux or total turnover rate, S is the rate of amino acid incorporation into protein or protein synthesis, C is the rate of amino acid oxidation, B represents the rate of amino acid release from endogenous protein breakdown, and I is the rate of exogenous intake. In the postabsorptive state, I equals 0 and B, therefore, equals Q. In general, routes of nonoxidative leucine disposal other than S are assumed to be negligible (12, 19). This statement is true in the pre- and postdialysis period when $S = Q - C$. During dialysis, when nonoxidative disposal of leucine must take into account dialysate loss, then $S = Q - (C + D)$ where D equals dialysate leucine loss. Leucine loss was measured in the spent dialysate and the loss was assumed to be linear during the entire 2.5 h of treatment. Net B or net leucine balance was derived from the difference between leucine protein incorporation (S) and leucine degradation (D); the latter being equal to Q or flux.

Leucine flux [Q] denotes movement of leucine into (rate of appearance [Ra]) and out of (rate of disappearance [Rd]) the metabolic pool. In steady state, $Ra = Rd$.

$$Q = [E_i/E_p - 1] \times i,$$

where E_i is [1-^{13}C] leucine enrichment in the infusate (100%), E_p is [1-^{13}C] leucine or [1-^{13}C] α-keto 150 caproate (KIC) enrichment in the plasma at isotopic plateau, and i is L-[1-^{13}C]leucine infusion rate [μmol/kg per h]. Leucine appearance rate into the plasma leucine pool was calculated from plasma [1-^{13}C]leucine enrichment (16), whereas leucine appearance rate into the whole body leucine pool was derived from plasma [1-^{13}C]KIC enrichment (20, 21).

The rate of $^{13}CO_2$ release from tracer leucine oxidation was calculated as follows: $F_{13_{CO_2}} = (F_{CO_2} \times E_{CO_2}/BW) \times (60 \times 41.6/100 \times 0.81)$.

F_{CO_2} is CO_2 production rate, E_{CO_2}, $^{13}CO_2$ enrichment in the expired gas at isotopic steady state, BW is body weight (kg). The constants 60 [min/h] and 41.6 [μmol/ml at standard temperature and pressure] convert F_{CO_2} from ml/min to μmol/h. The factor 100 changes atom percent excess from a percent to a fraction, and the factor 0.81 represents the fraction of $^{13}CO_2$ produced by [1-^{13}C]leucine oxidation released from the body bicarbonate pool into the expired gas. (We have not independently measured CO_2 recovery from the bicarbonate pool in the uremic subjects). The rate of leucine oxidation [C] is then (16)

$$C = F_{13_{CO_2}}[1/E_p - 1/E_i] \times 100 + D_{13_{CO_2}}[1/E_p - 1/E_i] \times 100,$$

where $D_{13_{CO_2}}$ is the amount of labeled CO_2 lost in the dialysate. $D_{13_{CO_2}}$ was not measured but was calculated as follows: we first estimated plasma CO_2 concentration from arterial P_{CO_2}. Plasma CO_2 concentration [mmol/liter] = arterial $P_{CO_2} \times 0.0301$, where 0.0301 is the solubility coefficient of CO_2. Then, $D_{13_{CO_2}}$ (μmol/kg/h) = [plasma CO_2 (mmol/liter) × Q_B (blood flow rate, liters/min) × 1,000 × 60] × [expired $^{13}CO_2$ (atom % excess)/100] divided by body weight (kg). Arterial P_{CO_2}. Q_B; and expired $^{13}CO_2$ enrichment were the measured parameters. In isotopic steady state, expired $^{13}CO_2$ enrichment reflects the circulating $^{13}CO_2$ enrichment (which is in equilibrium with body CO_2 and bicarbonate pool). Since dialysate bicarbonate represents loss from the same circulating pool, we assumed that the $^{13}CO_2$ enrichment of dialysate CO_2 at isotopic steady state was the same as that of the expired CO_2. Since product isotopic enrichment (in this case breath and dialysate $^{13}CO_2$) cannot exceed precursor isotopic enrichment, the above calculation, therefore, represents maximal potential $D_{13_{CO_2}}$ (see Tables III and IV). In other words, we are assuming complete plasma clearance of $^{13}CO_2$ during its passage through the dialyzer. In using dissolved CO_2 to calculate dialysate CO_2 loss, we reasoned that in the dialysate where carbonic anhydrase is absent and where contact between blood and dialysate is of transient duration (single-pass system where blood and dialysate flow rates were, respectively, 0.4 and 0.5

liters/min), hydration of CO_2 to H_2CO_3 will be delayed and, therefore, not in equilibrium with the dialysate bicarbonate.

To account for the changes in the natural abundance of $^{13}CO_2$ in expired gas during dialysis, additional studies were performed in four subjects in whom expired gases were collected as described below before, during, and after identical hemodialysis but in the absence of L-[1-^{13}C]leucine and $NaH^{13}CO_3$ administration. The average change in expired $^{13}CO_2$ enrichment of these four subjects at each dialysis and postdialysis period was subtracted from individual measured values of all study subjects during the same experimental period to yield corrected values for $^{13}CO_2$ enrichment that were then used in above equations to generate data on leucine oxidation and leucine protein incorporation.

Leucine and CO_2 measurement. [1-^{13}C]-enrichment of plasma leucine and plasma KIC was measured by gas chromatography mass spectrometry (22, 23) and $^{13}CO_2$ enrichment in the expired gas was measured by isotope ratio mass spectrometry as described previously (16).

Leucine content in the plasma and the spent dialysate was measured by an amino acid analyzer (model 6300; Beckman Instruments, Inc., Fullerton, CA) using norleucine as the internal standard (24). The data were analyzed using Waters Expert Ver. 6.2 1A software (Waters Instrs. Inc., Rochester, MN).

CO_2 production rate and collection of expired gas. CO_2 production rate [F_{CO_2}] was determined by a portable metabolic gas monitor [model MGMII; Utah Medical Products, Midvale, UT]. Subjects were given sufficient time to become familiarized with the equipment and the procedures. During measurement, a mouthpiece attached to a two-way valve was used. Expired gas was sampled by a small gas line leading from the mouthpiece to an infrared CO_2 sensor. Simultaneously, tidal volume and frequency of respiration were recorded by a pneumotachograph and expired ventilation by an ultrasonic flow transducer (25). Partial pressure of the inspired CO_2 was taken from that of the atmosphere. F_{CO_2} was calculated by standard equations and corrected for standard pressure and temperature, dry. Before each experiment, the equipment was calibrated with a standard reference gas consisting of 10% CO_2 and the flow transducer was checked by a calibrated syringe. Immediately after each measurement, the expired gas was collected into a Douglas bag and a sample was transferred anaerobically into sealed vacuum tubes (Venoject; Terumo Medical, Elkton, MO) for quantitation of $^{13}CO_2$ enrichment by isotope ratio mass spectrometry.

Arterial blood gas and serum bicarbonate. Arterial pH, P_{CO_2}, and serum bicarbonate concentration were measured before, at 60 and 90 min during dialysis, and immediately after dialysis. These measurements were determined to estimate CO_2 and bicarbonate fluxes during the dialysis procedure (see Table IV). Bicarbonate gain = (post-HCO_3^- − pre-HCO_3^-) × (BW × 0.4), where HCO_3^- represents serum bicarbonate levels post- and predialysis, BW is post dialysis body weight, and 0.4 represents bicarbonate distribution space or 40% of body weight. This 40% distribution appears to be appropriate for the pH ranges of our patients, 7.33–7.45 (26). Bicarbonate loss = [(pre-HCO_3^- + post-HCO_3^-)/2] × UF, where UF is ultrafiltration or fluid removal during dialysis. Net HCO_3^- gain is the difference between HCO_3^- gain and loss. If all the bicarbonate gained from the dialysate were to be titrated to CO_2, the predicted maximal amount of CO_2 release = (Net HCO_3^- gain/150) × 24, where 150 is the number of minutes in 2.5 h of dialysis and 24 is Avogadro's number, stating that 1 mol of CO_2 occupies a volume of 24 liters, and converts CO_2 from mmol to ml.

Statistical analysis. Data were stored in the Clinfo System of the Clinical Research Center of the University of Iowa College of Medicine; statistical analyses were completed using analysis of variance and Duncan's multiple comparison test.

Results

Fig. 1 depicts the mean plasma [1-^{13}C]leucine and [1-^{13}C]KIC enrichment before, during, and for 4 h after completion of dialysis. Each graph consists of 18 data points, 5 before initiation of dialysis, 5 during dialysis, and 8 after completion of dialysis. The atom percent excess of [1-^{13}C]leucine and [1-^{13}C]-KIC were stable during each period. The ratio of [1-^{13}C]-leucine and [1-^{13}C]KIC enrichment was 0.73. Table II summarizes leucine appearance rate into the plasma leucine and the total body leucine pools, the former was derived from plasma [1-^{13}C]leucine enrichment, and the latter derived from plasma [1-^{13}C]KIC enrichment (20, 21). The appearance rates were within normal ranges, stable during the entire 10.5 h of observation, and were unaffected by hemodialysis.

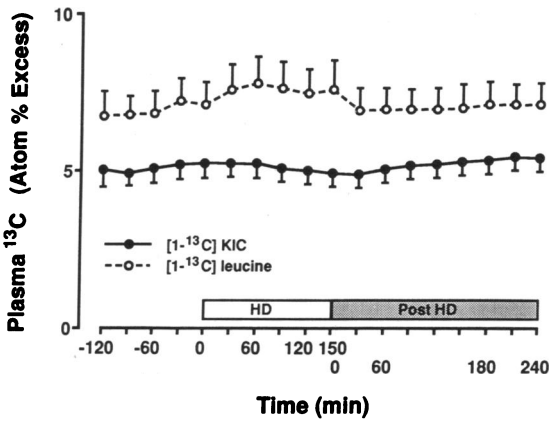

Figure 1. Plasma ^{13}C enrichment before, during, and after hemodialysis. Plasma [1-^{13}C]leucine and [1-^{13}C]KIC enrichment (mean±SEM) before, during, and after hemodialysis showing stable enrichment during the entire experiment. L-[1-^{13}C]leucine was administered by primed-constant infusion starting at −240 min and 2 h were allowed for isotopic equilibration before samples were taken. The times −120–0 refer to predialysis period HD and Post HD represent hemodialysis and posthemodialysis periods, respectively.

Table II. Leucine Appearance Rates

Dialysis period	Time	Leucine Ra: Into plasma leucine pool	Leucine Ra: Into total body leucine pool
	min	*μmol/kg per h*	
Before	0	86±27	118±31
During	60	78±25	114±29
	150	81±30	120±33
After	60	87±29	121±33
	120	85±25	115±29
	180	85±26	112±27
	240	83±22	109±25
ANOVA d.f.		6/124	6/124
F		0.952	0.317
P		0.264	0.927

Leucine appearance rates into the plasma leucine and total body leucine pools were derived, respectively, from plasma [1-^{13}C]leucine and plasma [1-^{13}C]KIC enrichment (see Methods). Values are presented as mean±SD. Statistical analysis was performed by one-way ANOVA and Duncan's multiple comparison test; the degree of freedom [d.f.], F, and P values are listed. All the values listed during different dialysis periods and different experiment times are not different from one another, and none of the values obtained during and after dialysis is different from that obtained before dialysis.

Table III summarizes expired $^{13}CO_2$ enrichment and appearance rates; the latter represents the amount of CO_2 derived from metabolism of L-[1-^{13}C]leucine. Despite an increased production of total CO_2 from 7,083 to 7,964 μmol/kg per h, the appearance rate of $^{13}CO_2$ decreased from 0.87 before to a nadir of 0.61 μmol/kg per h during dialysis. After completion of dialysis, total CO_2 production continued to be elevated, but expired gas $^{13}CO_2$ enrichment and $^{13}CO_2$ appearance rate rose concomitantly so that $^{13}CO_2$ appearance rates rose above predialysis values to 1.14 μmol/kg per h ($P < 0.001$).

Table IV summarizes bicarbonate and CO_2 fluxes during hemodialysis. The left half of the table lists ultrafiltration rate and changes in serum HCO_3^- and V_{CO_2} before and after dialysis whereas the right half documents HCO_3^- gain and loss as well as potential CO_2 and $^{13}CO_2$ losses during treatment. Mean HCO_3^- gain of 187 mmol is due to diffusion of bicarbonate from dialysate to blood and mean HCO_3^- loss of 21 mmol is a result of convective movement of bicarbonate from blood to dialysate consequent to ultrafiltration. The difference between these two processes resulted in a net HCO_3^- gain of 166 mmol during the 2.5 h of treatment. This bicarbonate gain would lead to a predicted increment in V_{CO_2} of 27 ml/min if all the bicarbonates were to titrate acid metabolites. Since only ~ 80% of the CO_2 produced is released, the corrected predicted rise in V_{CO_2} would be 21.6 ml/min. This predicted value is surprisingly close to the measured change in V_{CO_2} before and after dialysis, 20 ml/min. Mean potential CO_2 loss was 0.47 mmol/min or 457 μmol/kg per h whereas mean $^{13}CO_2$ loss was only 0.033 μmol/kg per h. Tolchin et al. (27) using acetate dialysate reported a CO_2 loss of 0.3 mmol/min. Since we calculated $^{13}CO_2$ loss in the dialysate (Table IV) as complete plasma clearance of $^{13}CO_2$ into the dialysate on each pass, D$^{13}CO_2$ was overestimated to some extend but was, nonetheless, only ~ 5% of the $^{13}CO_2$ appearance rate (Table III). The net result, then, was little overall effect on oxidation compared with labeled leucine carbon lost via the breath alone.

Fig. 2 illustrates changes in leucine flux, oxidation, protein incorporation, and net leucine balance during and after hemodialysis plotted as percentages of values obtained predialysis. Fig. 2 *A* shows virtually no fluctuations in leucine appearance rate into the total body leucine pool. By contrast, Fig. 2 *B* illustrates that leucine oxidation rate decreased to 75% of baseline during dialysis and rose to a peak of 130% 4 h after dialysis. Leucine protein incorporation rate, shown in Fig. 2 *C*, decreased to 83% of baseline during dialysis, rose in the first 2 h after-dialysis, but declined again 4 h later. Most importantly, net leucine balance, depicted as percent change from baseline in Fig. 2 *D*, showed a reduction by as much as 88% during dialysis compared with the predialysis value.

Table V presents the data of oxidative and nonoxidative leucine disposal in a detailed fashion by separating the intradialysis period into 60- and 150-min intervals and the postdialysis period into four hourly segments. During dialysis, leucine protein incorporation rate was significantly reduced, from 101 predialysis to 84 μmol/kg per h. This reduction was not due to increased leucine oxidation (which in fact was reduced) but was in part accountable by dialysate leucine loss (14.4 μmol/kg per h, ~ 15% of leucine appearance rate into the total body leucine pool). After dialysis, leucine protein incorporation rate returned towards normal level, only to become reduced 3 h later; this time it was accompanied by an increase in leucine oxidation. Although leucine oxidation rates in the pre- and postdialysis periods were derived solely from expired gas $^{13}CO_2$, oxidation rates during dialysis represent the sum of expired gas and dialysate $^{13}CO_2$ losses. The amount of dialysate $^{13}CO_2$ loss, however, was minimal, ~ 5% of the expired gas $^{13}CO_2$ appearance rate. Because of the combination of dialysate leucine loss and reduced leucine protein incorporation, net leucine balance was markedly reduced during dialysis.

Since all measurements during the predialysis period were stable, the values were averaged into one single predialysis mean to which intra- and postdialysis measurements were compared. Fig. 3 depicts the kinetics (μmol/kg per h, mean±SD) grouped into three periods. Leucine appearance into the total body leucine pool (Q), was stable, 118±31, 118±31, and 114±28, respectively, before, during, and after dialysis ($P = 0.813$). By contrast, leucine oxidation rate (C) fell from a pre-dialysis value of 17.3±7.8 to 13.8±7.8 during dialy-

Table III. Expired Gas $^{13}CO_2$ Enrichment and $^{13}CO_2$ Appearance Rate

Dialysis period	Time	FCO_2		$^{13}CO_2$ enrichment	$^{13}CO_2$ appearance
	min	*ml/min*	*μmol/kg per h*	*atom % excess*	*μmol/kg per h*
Before	0	193±46	7,083±1,688	0.0101±0.0042	0.87±0.27
During	60	217±48	7,964±1,761	0.0075±0.0026	0.74±0.19
	150	210±44	7,707±1,615	0.0063±0.0024*	0.61±0.24*
After	60	216±54	7,927±1,982	0.0084±0.0046	0.79±0.36
	120	212±47	7,781±1,725	0.0096±0.0035	0.92±0.31
	180	215±51	7,891±1,872	0.0110±0.0046	1.06±0.42
	240	219±56	8,027±2,055	0.0118±0.0053	1.14±0.45*
ANOVA d.f.		6/124		6/124	6/124
F		0.843		4.245	5.465
P		0.539		<0.001	<0.0001

Data are presented as mean±SD. FCO_2 and $^{13}CO_2$ appearance represent, respectively, CO_2 production rate and CO_2 derived from metabolism of [1-^{13}C]leucine. For ease of comparison FCO_2 is listed as ml/min as well as μmol/kg per h. The latter was derived using Avogadro's number stating that 1 mol of CO_2 occupies a volume of 24 liters. Statistical analysis was done using one-way ANOVA and Duncan's multiple comparison test; the degree of freedom (d.f.), F, and P values are listed. * Values that are significantly different from those obtained in the predialysis period.

Table IV. Bicarbonate Flux and Potential CO_2 and $^{13}CO_2$ Losses During Hemodialysis

Patient	Weight		Ultrafiltration	Serum HCO_3^-		ΔHCO_3^-	VCO_2		ΔVCO_2	HCO_3^- gain	HCO_3^- loss	Net HCO_3^- gain	Predicted $\uparrow VCO_2$	P_{CO_2}	Plasma CO_2	Potential		Expired gas $^{13}CO_2$	Potential $^{13}CO_2$ loss
	Before	After		Before	After		Before	After								CO_2 loss			
	kg		*liter*	*mmol/liter*			*ml/min*			*mmol*			*ml/min*	*mmHg*	*mmol/liter*	*mmol/min*	*μmol/kg per h*	*atom % excess*	*μmol/kg per h*
IA	78.5	77.0	1.50	27.7	31.3	3.6	178	181	3	111	44	67	11	39.1	1.18	0.47	366	0.0059	0.023
JD	76.0	74.6	1.40	24.0	30.2	6.2	260	278	18	185	38	147	24	31.6	0.95	0.38	306	0.0044	0.014
EB	83.6	82.1	1.50	23.5	29.8	6.3	162	192	30	207	40	167	27	40.6	1.22	0.49	358	0.0107	0.039
TD	41.3	40.3	1.00	28.4	35.2	6.8	141	158	17	110	32	78	12	51.1	1.54	0.62	923	0.0074	0.071
RF	88.4	87.9	0.50	18.8	28.3	9.5	217	255	38	334	12	322	52	36.7	1.10	0.44	300	0.0074	0.023
VM	56.4	55.8	0.60	20.5	29.1	8.6	157	182	25	192	15	177	28	35.0	1.05	0.42	452	0.0064	0.030
RS	51.9	53.2	−1.30	21.5	29.5	8.0	235	243	8	170	−33	203	33	37.0	1.11	0.44	496	0.0054	0.028
Mean	68.0	67.3	0.74	23.5	30.5	7.0	193	213	20	187	21	166	27	38.7	1.16	0.47	457	0.0068	0.033
SD	18.0	17.6	0.99	3.6	2.3	1.9	45	45	12	75	27	86	14	6.2	0.19	0.08	217	0.0020	0.019

Ultrafiltration refers to the amount of fluid removed during dialysis. The derivation of HCO_3^- gain and loss, predicted VCO_2 changes, plasma CO_2 concentration, and potential CO_2 and $^{13}CO_2$ losses are detailed in Methods. Patient RS had no convective HCO_3^- loss because he had medullary cystic kidney disease, usually lost weight in between dialysis, and required saline infusion during treatment.

sis and returned to 18.9±10.3 after dialysis ($P = 0.027$). The rate of leucine incorporation into protein (S) decreased from 101±26 before dialysis to 89±23 during dialysis, and 95±23 after dialysis ($P = 0.132$). Dialysate leucine loss (D), measured in the spent dialysate, was 14.4±6.2. Net leucine balance (*Net B*), derived from the difference between leucine protein incorporation rate and leucine derived from proteolysis declined from −17.3±7.8 before dialysis to −28.5±10.3 during dialysis, and reverted to −18.9±10.3 after dialysis ($P < 0.0001$).

Despite significant dialysate leucine loss, 14.4 μmol/kg per h, plasma leucine concentration (nmol/ml), however, was not reduced; 130±30 before dialysis, 131±26 during dialysis, and 164±28 after dialysis ($P = 0.014$).

Discussion

During primed-constant infusion of isotopic leucine, we failed to detect any evidence to implicate that hemodialysis stimulates protein degradation directly because plasma enrichment of [1-^{13}C]leucine and [1-^{13}C]KIC was not reduced (Fig. 1). Furthermore, leucine appearance rates into the plasma leucine pool, derived from plasma [1-^{13}C]leucine enrichment, was essentially stable during and for 4 h after dialysis (Table II). To circumvent the possibility that [1-^{13}C]leucine enrichment may underestimate leucine appearance into the whole body system (12), we used plasma [1-^{13}C]KIC enrichment or the "reciprocal pool" (21) method to estimate leucine appearance rate into the total body leucine pool. Again, plasma [1-^{13}C]KIC enrichment was remarkably constant during the entire period of observation. Since intracellular leucine pool in dialysis patients with adequate protein intake is normal (28, 29) and because the observed plasma [1-^{13}C]leucine to [1-^{13}C]KIC ratio of 0.73 is similar to that found in normal subjects (20), it is reasonable to assume that the measured values in our subjects reflected accurately body protein breakdown rates. If protein degradation was accelerated, we should have observed a reduction in plasma [1-^{13}C]leucine and [1-^{13}C]KIC enrichment and a rise in their respective flux rates. In our study, the labeled leucine was infused into the free amino acid pool, plasma in this instance. Dilution of the labeled leucine could occur either by leucine intake or degradation of body protein with release of unlabeled leucine. Since our experiment was done in the postabsorptive state, the magnitude of the isotopic dilution is proportional to proteolysis because intake = 0, and leucine, and essential amino acid, could be derived only from protein breakdown. On the basis of these leucine appearance rates, we conclude that hemodialysis, as performed in our unit, using a cuprophane membrane, bicarbonate dialysate, and treated water with zero bacteria colony counts, did not directly stimulate protein degradation acutely. The postdialysis blood urea nitrogen rebound must, therefore, be explained on the basis of urea dysequilibrium and redistribution (11).

In contrast to the marked stability of the leucine appearance rates, leucine oxidation and leucine protein incorporation rates fluctuated significantly throughout the study (Table V, Figs. 2 and 3). During dialysis, leucine oxidation was reduced. Such reduction, accompanied by increased CO_2 production, is perhaps best explained as a counter-regulatory response to augmented leucine loss in the dialysate. Alternatively, it may reflect a change in body fuel oxidation because of metabolism of glucose supplied in the dialysate. We did not measure plasma

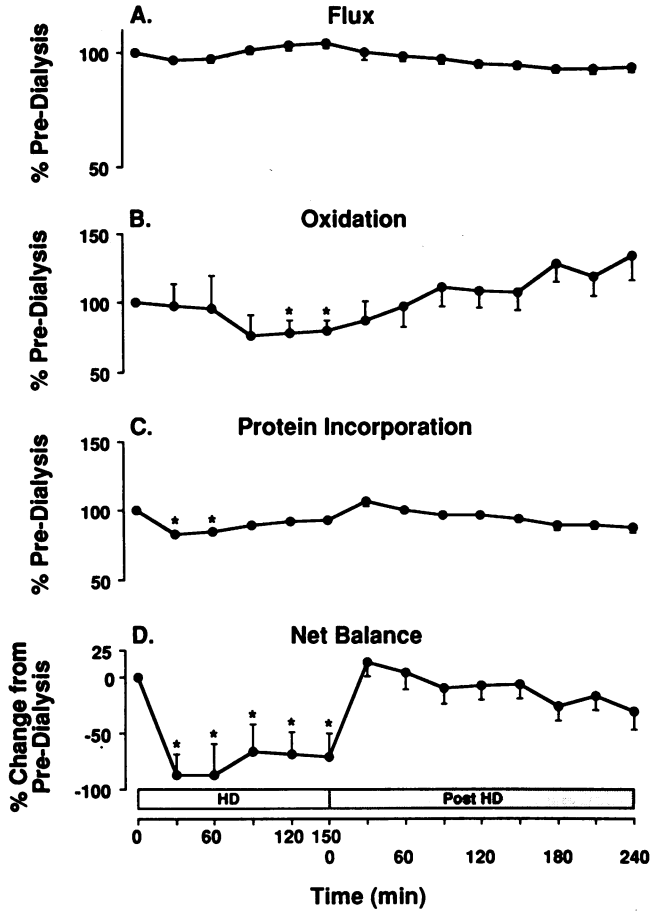

Figure 2. Changes in leucine kinetics during and after hemodialysis. Changes in leucine flux, oxidation, and protein incorporation were expressed as percent of predialysis values whereas changes in leucine net balance were plotted as percent change from predialysis. Results are listed as mean±SEM. *Values that are statistically different from those obtained during predialysis.

insulin but an insulin response would have been expected to diminish leucine appearance as well. After dialysis, the progressive rise in leucine oxidation rate was likely because of fasting. Although the non-oxidative leucine disposal in the pre- and postdialysis periods was limited to leucine protein incorporation, during dialysis leucine loss into the dialysate had to be accounted for. As a result, the calculated leucine protein incorporation rate was reduced during dialysis. After dialysis, especially 3–4 h later, leucine incorporation rate was also lower; but the reduction at this time was attributed to increased leucine oxidation. These postdialysis changes are consistent with metabolic status of prolonged fasting, a situation created by our experimental design.

Because of dialysate leucine loss and reduced protein incorporation, net leucine balance became markedly more negative during dialysis. Thus, even though hemodialysis did not stimulate protein degradation directly, the procedure, nevertheless, was a net catabolic event in the sense that protein synthesis was reduced and a significant quantity of amino acids was lost in the dialysate. Whether the reduction in protein synthesis is causally related to amino acid loss in the dialysate is not clear. On the basis of the knowledge that average leucine content of protein in human and other mammalian muscles is ~ 7.8% (16), the values of leucine flux can be used to estimate whole body protein turnover. A reduction of net leucine balance of −12.3 μmol/kg per h from predialysis to dialysis period (Table V) would be equivalent to a reduction of protein synthesis by 5.8 g in a 4-h dialysis session for a 70-kg man.

Table IV provides data indicating that despite bicarbonate and CO_2 fluxes across the membrane, dialysis only minimally alters the results of the leucine oxidation. In our patients, there was a net bicarbonate gain, which was used in the titration of acids and production of CO_2, resulting in a rise in V_{CO_2}. Such increase in V_{CO_2} would dilute the expired gas $^{13}CO_2$ enrichment but should not alter the absolute $^{13}CO_2$ production rate or net leucine oxidative loss, which is the product of expired $^{13}CO_2$ enrichment and total volume of irreversibly carbon loss,

Table V. Oxidative and Nonoxidative Leucine Disposal

Dialysis period	Time	Leucine disposal: Oxidation (C)	Dialysate loss	Protein incorporation (S)	Net leucine balance
	min			*μmol/kg per h*	
Before		17.3±7.8		101±26	−17.3±7.8
During	60	14.9±6.0	14.4±6.2	84±20*	−29.6±9.9*
	150	13.0±7.0*	14.4±6.2	92±24	−27.7±11.9*
After	60	16.3±9.8		104±27	−16.3±9.8
	120	18.2±9.4		97±22	−18.2±9.4
	180	20.3±12.3		91±20	−20.3±12.3
	240	20.7±10.1		89±21	−20.7±10.1
ANOVA d.f.		6/124		6/124	6/124
F		1.661		1.461	4.780
P		0.137		0.197	0.002

Although leucine oxidation rate and leucine loss into the dialysate were directly measured, leucine protein incorporation rate and net leucine balance were calculated (see Methods). All values are listed as mean±SD. Statistical analysis was performed with ANOVA and Duncan's multiple comparison test; the degrees of freedom (d.f.), *F*, and *P* values are listed. * Values that are significantly different from those obtained during the predialysis period.

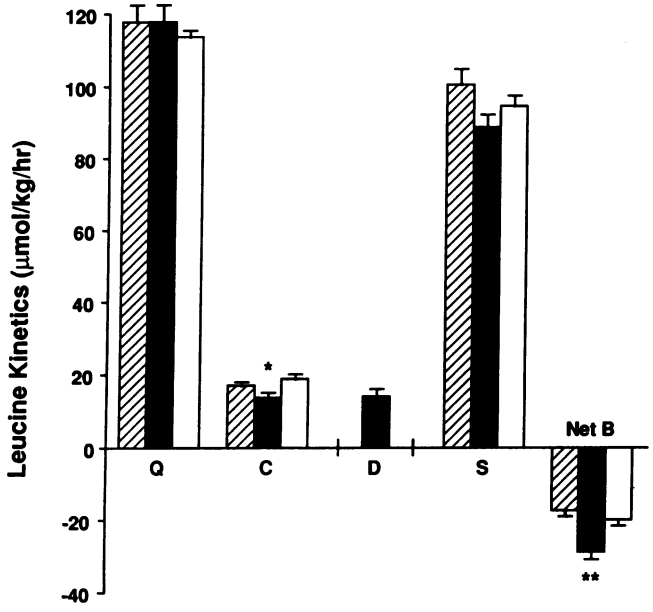

Figure 3. Leucine kinetics before, during, and after hemodialysis. Leucine turnover, presented as mean±SEM, as derived from plasma [1-^{13}C]KIC and dialysate and expired $^{13}CO_2$ losses, were pooled into three separate periods, before (▨), during (■), and after (□) dialysis. *Q*, leucine appearance rate into the total body leucine pool, equal to leucine released from protein degradation; *C*, leucine oxidation rate; *D*, leucine loss into dialysate; *S*, leucine protein incorporation rate; and *Net B*, leucine net balance equal to *S*−*Q*. One-way ANOVA and Duncan's multiple test were used to assess the differences among the three periods. $*P = 0.01$ and $**P = 0.001$ as compared with predialysis values.

V_{CO_2} in this instance. The same table also provides information on potential maximal amounts of dialysate $^{13}CO_2$ loss, which is 0.033 μmol/kg per h. We have taken this small amount of dialysate $^{13}CO_2$ loss into consideration in the calculation of leucine oxidation rates (see Methods). Since $D^{13}CO_2$ loss is only a small fraction, ~ 5% of total expired $^{13}CO_2$ loss, the inclusion of such item did not alter the results of leucine oxidation and protein incorporation rates.

We did not measure labeled bicarbonate dialysate loss but have calculated its potential loss on the basis of the worse scenario that plasma $H^{13}CO_3^-$ is totally cleared during each pass through the dialyzer and that the direction of flux is entirely from the blood to the dialysate. Such calculations increased the estimated leucine oxidation rate and yielded a further reduction in leucine protein incorporation rate and a greater negative net leucine balance during dialysis. Because the calculations did not alter the overall conclusions of this work and are fraught with assumptions, the derived data are not included in this paper. It should be emphasized that during bicarbonate dialysis, the major route of bicarbonate/CO_2 loss is via the lungs, 533 mmol/h, and not the dialyzer, which lost only 31 mmol/h (Tables III and IV).

These findings are consistent with the report of Borah et al. (9), documenting that nitrogen balance is always less on the day of dialysis, and with our own data showing that lengthening the interdialytic interval tends to improve nitrogen balance (10). By measuring arterio-venous amino acid gradient and total blood flow across the leg, Gutierrez and colleagues (14) found that 150 min of sham hemodialysis in normal control subjects led to increased amino acid release, suggesting accelerated protein breakdown. If our patients had a twofold increase in amino acid release, we should have observed a marked reduction in plasma [1-^{13}C]leucine and [1-^{13}C]KIC enrichment and an increase in the calculated leucine flux, and we did not. Moreover, we found changes during dialysis whereas Gutierrez and associates (14) detected changes after dialysis. These discrepancies are difficult to reconcile and may be related to differences in the techniques used.

Our inability to find a change in plasma isotopic dilution could not be due to technical difficulty as the method of primed-constant infusion of a stable isotope is sufficiently sensitive to detect as little as a 2% change in flux rate (12, 16). Recycling of labeled leucine may mask an increase in leucine flux, although label recycling is usually not a significant problem for the first 6–8 h of isotope infusion. Our study was 10.5-h long; thus it is possible that we might have missed a small increase in flux in the latter part of the experimental period.

The amount of leucine lost into the dialysate, 2.26 mmol or 296 mg, was similar to that reported by Wolfson et al. (6). Despite this removal, serum leucine level was not reduced during dialysis and actually rose modestly after dialysis. The maintenance of a constant leucine level must be regulated by a balance between catabolism and anabolism. In our study, leucine protein incorporation rate, on the basis of plasma [1-^{13}C]KIC enrichment, was reduced from a predialysis value of 101 to a nadir of 84 μmol/kg per h during dialysis (Table V). This magnitude of reduction would actually provide 2.86 mmol of free leucine on the basis of a mean body weight of 67.3 kg in our subjects. This amount is very close to the measured dialysate loss, 2.26 mmol. Thus, maintenance of a stable serum leucine level in our study was the result of reduced leucine incorporation into protein.

Finally, it should be emphasized that our data pertain only to acute changes during and immediately after dialysis and do not necessarily exclude the possibility that repeated cytokine release with hemodialysis might enhance protein catabolism over a long period of time (30, 31).

In summary, we found that hemodialysis using a cuprophane membrane and bicarbonate dialysate did not increase protein degradation because leucine appearance rates were stable. The procedure, nevertheless, was a net catabolic event because protein synthesis was reduced and there was a net loss of amino acids into the spent dialysate.

Acknowledgments

We thank the staff of the Dialysis Unit for patient care; Steve Baker, David Tatman, Howard Christopherson, and Richard Berger for technical assistance; and Sarah Parker for secretarial help.

This study was supported by an award from Baxter's Extramural Grant Program, National Institutes of Health grants RR55 (General Clinical Research Center), and RR954 (Mass Spectrometry Resource Center), and Amgen Clinical Grants Program.

References

1. Bergstrom, J., A. Alvestrand, and A. Gutierrez. 1986. Acute and chronic metabolic effects of hemodialysis. *In* Immune and Metabolic Aspects of Therapeutic Blood Purification Systems: Proceedings of the International Symposium on Purification Systems. L. Smeby, S. Jorstead, and T.-E. Wideroe, editors. Karger, Basel, Switzerland/New York. 254–273.

2. Heidland, A., R. M. Schaefer, E. Heidbreder, and W. H. Horl. 1988. Cata-

bolic factors in renal failure: therapeutic approaches. *Nephrol. Dial. Transplant.* 3:8–16.

3. Heidland, A., W. H. Horl, N. Heller, H. Heine, S. Neumann, and E. Heidbreder. 1983. Proteolytic enzymes and catabolism: enhanced release of granulocyte proteinases in uremic intoxication and during hemodialysis. *Kidney Int. Suppl.* 24:S27–S36.

4. Kopple, J. D., M. E. Swendseid, J. H. Shinaberger, and C. Y. Umezawa CY. 1973. The free and bound amino acids removed by hemodialysis. *Trans. Am. Soc. Artif. Intern. Organs.* 19:309–313.

5. Tepper, T., G. K. Van Der Hem, H. G. Klip, and A. J. M. Donker. 1981. Loss of amino acids during hemodialysis. Effect of oral essential amino acid supplementation. *Nephron.* 29:25–29.

6. Wolfson, M., M. R. Jones, and J. D. Kopple. 1982. Amino acid losses during hemodialysis with infusion of amino acids and glucose. *Kidney Int.* 21:500–506.

7. Farrell, P. C., and P. W. Hone. 1980. Dialysis-induced catabolism. *Am. J. Clin. Nutr.* 33:1417–1422.

8. Ward, R. A., M. J. Shirlow, J. M. Hayes, G. V. Chapman, and P. C. Farrell. 1979. Protein catabolism during hemodialysis. *Am. J. Clin. Nutr.* 32:2443–2449.

9. Borah, M. F., P. Y. Schoenfeld, F. A. Gotch, J. A. Sargent, M. Wolfson, and M. H. Humphreys. 1978. Nitrogen balance during intermittent dialysis therapy of uremia. *Kidney Int.* 14:491–500.

10. Lim, V. S., and M. J. Flanigan. 1989. The effect of interdialytic interval on protein metabolism: evidence suggesting dialysis-induced catabolism. *Am. J. Kidney Dis.* 14:96–100.

11. Pedrini, L. A., S. Zereik, and S. Rasmy. 1988. Causes, kinetics and clinical implications of post-hemodialysis urea rebound. *Kidney Int.* 34:817–824.

12. Bier, D. M. 1989. Intrinsically difficult problems: the kinetics of body proteins and amino acids in man. *Diabetes Metab. Rev.* 5:111–132.

13. Gotch, F. A. 1986. Kinetics of hemodialysis. *Artif. Organs.* 10:272–281.

14. Gutierrez, A., A. Alvestrand, J. Wahren, and J. Bergstrom. 1990. Effect of in vivo contact between blood and dialysis membranes on protein catabolism in humans. *Kidney Int.* 38:487–494.

15. Lim, V. S., M. J. Flanigan, and J. Fangman. 1990. Effect of hematocrit on solute removal during high efficiency hemodialysis. *Kidney Int.* 37:1557–1562.

16. Matthews, D. E., K. J. Motil, D. K. Rohrbaugh, J. F. Burke, V. R. Young, and D. M. Bier. 1980. Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-13C] leucine. *Am. J. Physiol.* 238:E473–E479.

17. Motil, K. J., D. E. Matthews, D. M. Bier, J. F. Burke, H. N. Munro, and V. R. Young. 1981. Whole-body leucine and lysine metabolism: response to dietary protein intake in young men. *Am. J. Physiol.* 240:E712–E721.

18. Matthews, D. E., D. M. Bier, M. J. Rennie, R. H. T. Edwards, D. Halliday, D. J. Millward, and G. A. Clugston. 1981. Regulation of leucine metabolism in man: a stable isotope study. *Science (Wash. DC).* 214:1129–1131.

19. Bier, D. M., D. E. Matthews, and V. R. Young. 1985. Interpretation of amino acid kinetic studies in the context of whole-body protein metabolism. *In* Substrate and Energy Metabolism. J. S. Garrow and D. Halliday, editors. John Libbey, London. 27–36.

20. Matthews, D. E., H. P. Schwarz, R. D. Yang, K. J. Motil, V. R. Young, and D. M. Bier. 1982. Relationship of plasma leucine and a-ketoisocaproate during L-[1-^{13}C] leucine infusion in man: a method for measuring human intracellular leucine tracer enrichment. *Metabolism* 31:1105–1112.

21. Schwenk, W. F., B. Beaufrere, and M. W. Haymond. 1985. Use of reciprocal pool specific activities to model leucine metabolism in humans. *Am. J. Physiol.* 249:E646–E650.

22. Matthews, D. E., E. Ben-Galim, and D. M. Bier. 1979. Determination of stable isotopic enrichment in individual plasma amino acids by chemical ionization mass spectrometry. *Anal. Chem.* 51:80–84.

23. Schwarz, H. P., I. E. Karl, and D. M. Bier. 1980. The a-keto acids: simplified derivatization for physiological samples and complete separation as quinoxalinols by packed column gas chromatography. *Anal. Biochem.* 108:360–366.

24. Cohen, S. A., T. L. Tarvin, and B. A. Bidlingmeyer. 1984. Analysis of amino acids. *Am. Lab. (Shelton).* 16:48–59.

25. Westenskow, D. R., C. A. Cutler, and W. D. Wallace. 1984. Instrumentation for monitoring gas exchange and metabolic rate in critically ill patients. *Crit. Care Med.* 12:183–187.

26. Adrogue, H., J. Brensilver, J. Cohen, and N. Madias. 1983. Influence of steady-state alterations in acid-base equilibrium on the fate of administered bicarbonate in the dog. *J. Clin. Invest.* 71:867–883.

27. Tolchin, N., J. Roberts, and E. Lewis. 1978. Respiratory gas exchange by high-efficiency hemodialyzers. *Nephron.* 21:137–145.

28. Alvestrand, A., P. Furst, and J. Bergstrom. 1982. Plasma and muscle free amino acids in uremia: influence of nutrition with amino acids. *Clin. Nephrol.* 18:297–305.

29. Alvestrand, A., P. Furst, and J. Bergstrom. 1983. Intracellular amino acids in uremia. *Kidney Int. Suppl.* 24:S9–S16.

30. Lindsay, R. M., and E. Spanner. 1989. A hypothesis: the protein catabolic rate is dependent upon the type and amount of treatment in dialyzed uremic patients. *Am. J. Kidney Dis.* 13:382–389.

31. Luger, A., J. Kovaric, H. K. Stummvoll, A. Urbanska, and T. A. Luger. 1987. Blood-membrane interaction in hemodialysis leads to increased cytokine production. *Kidney Int.* 32:84–88.