

# Muscle Protein Turnover and Glucose Uptake in Acutely Uremic Rats

## EFFECTS OF INSULIN AND THE DURATION OF RENAL INSUFFICIENCY

ANDREW S. CLARK and WILLIAM E. MITCH, *Department of Medicine, Harvard Medical School and The Brigham and Women's Hospital, Boston, Massachusetts 02115*

**ABSTRACT** Acute renal failure (ARF) in rats is associated with increased amino acid release from peripheral tissues and insulin resistance. To study whether abnormal protein and carbohydrate metabolism are linked in ARF, the effects of insulin on net muscle protein degradation (T) and on glucose uptake were measured in the perfused hindquarters of paired ARF and sham-operated (SO) rats. The basal rate of T increased 40% after 24 and 98% after 48 h of ARF. Insulin was less effective in decreasing T in ARF (−79% SO vs. −22% ARF 24 h and −64% SO vs. −23% ARF 48 h;  $P < 0.01$ ). Protein synthesis (PS) and protein degradation (PD) were measured independently in incubated epitrochlearis muscles; the increase in T after 24 h of ARF was due specifically to increased PD, while PS was unchanged. At this stage, insulin was less effective in decreasing PD in ARF (−10% ARF vs. −23% SO;  $P < 0.02$ ), although PS responded normally. After 48 h of ARF, the further increment in T was caused by the additional appearance of depressed basal and insulin-stimulated PS. This was confirmed in the perfused hindquarter ( $26 \pm 3$  ARF vs.  $38 \pm 3$  SO, basal;  $54 \pm 5$  ARF vs.  $73 \pm 7$  SO, insulin-stimulated, nmol phenylalanine/g per h;  $P < 0.05$ ).

Although basal glucose uptake by hindquarters of ARF and SO rats was comparable, insulin-stimulated glucose uptake was 33% less at 24 and 44% less after 48 h of ARF. After 48 h of ARF, lactate and alanine release were increased and net glycogen synthesis in muscle was depressed. These abnormalities were even more apparent in the presence of insulin. Inefficient glucose utilization, estimated as the ratio of lactate

release to glucose uptake, was correlated with T ( $r = +0.78$ ;  $P < 0.001$ ).

In conclusion, after 24 h of ARF, both increased PD and altered glucose utilization could be detected. After 48 h of ARF, T increased further because PS was depressed. At this time, glucose utilization was clearly abnormal and the results suggest that abnormal net protein degradation in ARF may be a consequence of defective glucose utilization.

## INTRODUCTION

Acute renal failure (ARF)<sup>1</sup> leads to a constellation of metabolic abnormalities by mechanisms that are poorly understood. For example, in experimental animals with ARF, there is increased hepatic production of glucose and urea (1–4), resistance to insulin-stimulated glucose and amino acid uptake by muscle (5, 6) and accelerated release of amino acids from peripheral tissues *in situ* and *in vitro* (4). Since insulin affects muscle metabolism in normal animals by increasing the rate of protein synthesis and decreasing the rate of protein degradation (7), it seemed possible that the metabolic abnormalities of acute uremia could be linked to an abnormal response to insulin. It would be important to determine whether muscle protein turnover is abnormal in ARF and whether it responds abnormally to insulin, since excessive net protein degradation would expand further the accumulated waste products derived from protein catabolism.

Although changes in the components of muscle pro-

Address reprint requests to Dr. Mitch.

Received for publication 6 October 1982 and in revised form 17 March 1983.

<sup>1</sup> Abbreviations used in this paper: ARF, acute renal failure; ECSA, extracellular specific radioactivity; ICSA, intracellular specific activity; L/R, ratio of lactate release to glucose uptake; SO, sham operated.

tein turnover and their response to insulin have not been documented in acutely uremic rats, Garber (8) reported that protein turnover in incubated epitrochlearis muscles of chronically uremic rats was less responsive to insulin than it was in muscles from control rats. In those experiments, the basal rates of release of the major gluconeogenic and ureagenic precursors, alanine and glutamine, were higher from muscles of uremic rats than from control rats, even though their release could be more readily suppressed by incubating with insulin. However, the regulation of protein turnover in ARF may be quite different from that in chronic uremia because muscle uptake of glucose and amino acids is insensitive to insulin in acutely uremic rats (5, 6).

In the present study, we sought to determine the responsiveness of skeletal muscle from acutely uremic rats to insulin. Thus, we measured the effects of insulin on glucose uptake and on net protein degradation in the isolated, perfused hindquarter from rats with acute uremia at different stages of severity. Because the perfused hindquarter is composed of several tissues besides muscle (9), the components of protein turnover also were assessed in incubated epitrochlearis muscles from rats of comparable age to confirm that abnormalities uncovered in the hindquarter also were present in muscle.

## METHODS

**Animals.** Male Sprague-Dawley rats weighing 180–220 g (Charles River Breeding Laboratories, Inc., Wilmington, MA) were maintained on a 12-h-light/12-h-dark cycle for at least 3 d before being studied. They were allowed to drink water ad lib. and were fed RMH 1000 chow (Agway Country Foods, Agway Inc., Syracuse, NY) containing 14% protein until ~4 h before they underwent sham operation (SO) or bilateral ureteral ligation to produce ARF. Adult rats were used in all studies because muscle protein degradation scarcely changes when they are starved 48 h (10–12), whereas it changes markedly in immature rats after only short periods of starvation. Therefore, changes in protein degradation observed in rats with ARF would not be confused with changes caused by starvation. Animals were paired by weight and randomly assigned to undergo bilateral ligation (ARF) or SO after anesthesia with 5 mg i.p./100 g body wt of sodium pentobarbital. After surgery, rats were placed in individual wire-bottomed cages to prevent coprophagia and were deprived of food and water for periods of either 20–24 or 44–48 h before being studied.

**Materials.** Radiolabeled compounds were obtained from Schwartz/Mann Div., Becton, Dickinson & Co., Orangeburg, NY, and reagent grade chemicals from Fisher Scientific Co., Pittsburgh, PA. Enzymes were obtained from Sigma Chemical Co., St. Louis, MO, and crystalline bovine insulin from Eli Lilly & Co., Indianapolis, IN. Bovine serum albumin obtained from Miles Laboratories, Inc., Elkhart, IN, was dissolved in Krebs-Henseleit bicarbonate buffer and dialyzed against a 10-fold excess of the buffer for 24 h. The dialyzed albumin solution was then stored at  $-20^{\circ}\text{C}$  until used in hindquarter perfusions.

**Hindquarter perfusion.** The perfusion procedure was similar to that used previously (4, 13), based on the technique of Ruderman et al. (14). Perfusates of 150 ml each were prepared freshly before each experiment and gassed with 95%  $\text{O}_2$ /5%  $\text{CO}_2$ . The media consisted of aged, washed human erythrocytes (hematocrit, 25%) in Krebs-Henseleit bicarbonate buffer, prefiltered albumin (final concentration, 3 g/dl), 10 mM glucose, 0.5 mM phenylalanine, and other amino acids at concentrations found in rat plasma (15). In experiments assessing the effects of insulin, 500  $\mu\text{U/ml}$  insulin were added after the equilibration period (7, 16).

The experiments were planned so that the hindquarter from an ARF rat was perfused simultaneously with the previously paired SO rat. The perfusate was recirculated in the perfusion chambers for 15 or more minutes while it was gassed and heated to  $37^{\circ}\text{C}$ . The pH was then checked and, if necessary, adjusted to pH 7.4; the perfusion was begun with a flow rate of 12–14 ml/min (Gilmont Flow Meter, Gilmont Instruments, Inc., Great Neck, NY). After placement of the aortic and vena caval cannulae, the spinal cord was clamped to avoid high perfusion pressures occurring as the animal died. The initial 20–30 ml of media were discarded and the remainder was recirculated continuously for a 30-min equilibration period and a subsequent 60-min experimental period. Experiments were discarded when there was evidence of inadequate perfusion, e.g., a flow rate  $<10$  ml/min, a rising perfusion pressure, or lactate release 8  $\mu\text{mol/g}$  per h (4, 14).

Aliquots of perfusate were obtained at 30 and 90 min for subsequent determination of glucose, lactate, tyrosine, phenylalanine, and alanine concentrations. Rates of uptake or release of these compounds were calculated from changes in their concentration and the volume of the perfusate. They were expressed per gram of muscle perfused, using the relationships between body weight and muscle perfused from ARF and SO rats determined previously (4).

In separate experiments, net flux of glucose into muscle glycogen was determined as described by Richter et al. (16). The rate of incorporation of  $[\text{U}-^{14}\text{C}]\text{D-glucose}$  into glycogen in soleus, extensor digitorum longus, and red and white gastrocnemius muscle was measured during 60 min of perfusion in the presence and absence of insulin.

**Epitrochlearis muscle incubation.** The epitrochlearis muscle was used to study the effect of uremia on muscle protein synthesis and degradation because its weight (28–32 mg) in adult rats is comparable to that of soleus and extensor digitorum longus muscles of immature rats. In contrast to these latter muscles, the epitrochlearis has a large surface relative to its weight (17) and therefore, there is more equal diffusion of substrates, a critical factor necessary for assessing the components of protein turnover in vitro (18). The epitrochlearis contains ~10–15% slow-twitch, red; 20% fast-twitch, red; and 65% fast-twitch, white muscle fibers (17). In previous studies, we had found that rates of protein synthesis and degradation in the epitrochlearis of fed and starved rats are quite similar to those measured by the perfused hindquarter technique (19).

After the rats were anesthetized, the muscles were dissected free, blotted, weighed, and placed in a flask containing 3 ml of Krebs-Henseleit bicarbonate buffer, 10 mM glucose, and 0.5 mM phenylalanine ( $\pm 0.05 \mu\text{Ci/ml}$   $[\text{U}-^{14}\text{C}]\text{phenylalanine}$ ). Experiments were discarded if the muscle weighed  $>40$  mg. The flasks were stoppered, gassed for 3 min with 95%  $\text{O}_2$ /5%  $\text{CO}_2$ , and placed in a rotating 60-cycle/min bath maintained at  $37^{\circ}\text{C}$ . After 30 min of preincubation, the muscles were removed, blotted, and transferred to fresh incubation flasks containing 3 ml of the same

media  $\pm 1$  m U/ml insulin as indicated, and incubated for an additional 2-h experimental period. This concentration of insulin was studied because it has been shown to cause an increment in glucose uptake comparable to that produced in the perfused hindquarter by 0.5 m U/ml insulin (20).

**Protein synthesis.** As shown by Li et al. (18), incorporation of radiolabeled amino acids into muscle protein will vary depending on changes in the intracellular specific radioactivity (ICSA) of the amino acid. With a high concentration of phenylalanine in the media, there is rapid equilibration of the specific activity of phenylalanine between the extracellular and intracellular compartments of muscle in both fed and starved rats (7, 19) but because amino acid transport is abnormal in muscle from uremic rats (6), we measured the ICSA of the amino acids being studied (phenylalanine and tyrosine) and compared them with the extracellular specific radioactivity (ECSA) of perfused and incubated muscle at the beginning and end of the experimental periods. Phenylalanine and tyrosine were chosen because muscle neither synthesizes nor degrades these amino acids (7, 18). To calculate the ICSA, previously determined values (4) for water content and extracellular volume of perfused muscle were used. The water content of the epitrochlearis muscle ( $71.2 \pm 1.3\%$  wt for ARF;  $69.5 \pm 1.4\%$  wt for SO;  $n = 6$ ) was measured by drying to constant weight; its extracellular volume ( $^{14}\text{C}$ -insulin space) was determined to be  $26.7 \pm 1.6\%$  wt for muscles from ARF rats and  $23.6 \pm 2.7\%$  wt for SO rats ( $n = 5$ ). As shown in Fig. 1, the ICSA and ECSA of phenylalanine were virtually identical after 30 min of perfusion of hindquarters or incubation of epitrochlearis muscles of rats with ARF and there was no significant change in the ratio during the experimental period. The ICSA and ECSA of tyrosine also equilibrated rapidly in incubations of epitrochlearis muscle from rats with ARF and similar findings for phenylalanine and tyrosine were obtained from SO rats (data not shown). When the extracellular concentration of tyrosine was  $494 \mu\text{M}$ , the intracellular concentration of tyrosine in uremic muscle after 30 min of incubation was  $420 \pm 30 \mu\text{M}$  compared with  $427 \pm 32 \mu\text{M}$  in muscles from SO rats. These findings confirm in muscle of acutely uremic rats that with a high extracellular concentration of phenylalanine or tyrosine there is rapid equilibration of the intracellular compartment with extracellular amino acids (7, 19). Determining this characteristic permitted us to calculate synthesis based on the ECSA of phenylalanine, thus considerably reducing the number of analyses.

To determine protein synthetic rates in the hindquarter

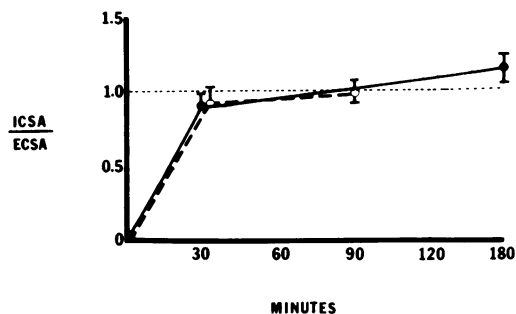


FIGURE 1 Mean ( $\pm$ SEM) values for the ratio between the ICSA and ECSA of phenylalanine measured in the perfused hindquarter (●) and in incubated epitrochlearis muscles (○) of ARF rats. The ECSA and ICSA equilibrated after 30 min and did not change significantly during the experiments.

experiments,  $0.05 \mu\text{Ci/ml}$  of  $[\text{U-}^{14}\text{C}]$ phenylalanine were added to the perfusate and samples of perfusate and gastrocnemius muscle were obtained after 30 and 90 min of perfusion. The perfusate was deproteinized with an equal volume of 10% TCA and the ECSA of phenylalanine was determined. The muscle sample was weighed and immediately homogenized in 3 ml of 10% TCA, washed successively with 10% TCA and ethanol/ether (1:1) and dissolved in Soluene (Packard Instrument Co., Downers Grove, IL) for scintillation counting with correction for quenching using an external standard. The difference in the radioactivity incorporated into muscle protein during the experimental period divided by the average ECSA was used to estimate the rate of protein synthesis (7, 12).

To determine the rate of protein synthesis in experiments using the epitrochlearis muscle,  $0.05 \mu\text{Ci/ml}$  of  $[\text{U-}^{14}\text{C}]$ phenylalanine was added to the media and one muscle was harvested, washed, and dissolved in Soluene, as described above, for determination of radioactivity after 30 min of the preincubation period; the contralateral muscle was treated similarly at the end of the experimental period. As with the perfusion experiments, the difference in incorporated radioactivity was then divided by the ECSA of phenylalanine and expressed per gram of muscle weight to calculate the rate of synthesis. This procedure corrected for variable rates of  $[\text{U-}^{14}\text{C}]$ phenylalanine incorporated during the period when the ICSA of phenylalanine was rising.

To assess the effect of insulin on protein synthesis in the epitrochlearis muscle, the  $[\text{U-}^{14}\text{C}]$ phenylalanine incorporated into protein was determined without added insulin in eight muscles during the initial 30 min. The rate of incorporation was quite uniform in ARF and SO rat muscle (coefficient of variation  $< 7\%$ ). This initial amount of  $[\text{U-}^{14}\text{C}]$ phenylalanine was subtracted from that incorporated during paired experiments in which one muscle was incubated without insulin and the contralateral muscle with 1 mU/ml insulin.

**Protein degradation.** In the perfused hindquarter experiments, net rates of protein degradation were measured from the change in the perfusate content of tyrosine (12). In experiments with incubated epitrochlearis muscle, total protein degradation was measured by incubating with 0.5 mM cycloheximide (10, 19) and calculating the rate of release of tyrosine into the incubation media during the 2-h experimental period.

**Analytic procedures.** Phenylalanine and tyrosine were measured fluorometrically (21–23); lactate, alanine, and urea were measured by enzymatic assays as previously described (4); and glucose was measured with a Glucose Analyzer 2 (Beckman Instruments, Inc., Fullerton, CA). There was no significant difference in the results obtained with this analyzer and those obtained with glucose oxidase and peroxidase (24). Muscle glycogen was isolated by dissolving the tissue in 30% potassium hydroxide and subsequent precipitation in ethanol, as described by Good et al. (25). Creatinine was measured with a Creatinine Analyzer (Beckman Instruments, Inc.). Arterial blood pH was measured with an Instrumentation Laboratory, Inc. (Lexington, MA) model 313 pH/Blood Gas Analyzer. The ATP and creatine phosphate content of muscle were measured in freeze-clamped samples by enzymatic methods (26).

During measurement of serum insulin concentrations by radioimmunoassay (27), several of the samples were observed to form a gel. Since gel formation would interfere with the radioimmunoassay, subsequent samples were mixed with polyethylene glycol (28) to remove large proteins before measuring immunoreactive insulin. Insulin recovery from sera treated in this fashion is  $95 \pm 2\%$  (28).

Statistical differences in results were tested by analysis of variance (29), except for changes in protein synthesis and degradation induced by insulin, the effects of insulin on the rate of alanine release from the perfused hindquarter, and changes in net muscle glycogen synthesis during perfusion. These results were examined by the *t* test.

## RESULTS

Table I shows initial weights, changes in weight, and values of arterial pH and blood chemistries for rats with ARF and for SO control rats fasted for 24 and 48 h after surgery. Weight loss by SO rats was significantly greater than that of ARF rats. This presumably was due to the difference in fluid excretion of the two groups. As expected, the concentrations of urea and creatinine was increased significantly in the uremic rats. After 48 h, the arterial blood pH was slightly lower in ARF rats and plasma potassium was significantly higher than in SO rats. The concentrations of serum glucose of ARF rats was not different statistically from that of SO rats after 24 or 48 h of fasting. The serum insulin concentrations also were not different statistically, presumably because insulin secretion was not stimulated and possibly because the kidneys retained some ability to degrade insulin.

The content of ATP and creatine phosphate measured in freeze-clamped gastrocnemius muscle obtained *in situ* from four fed, anesthetized rats were  $4.6 \pm 0.2$  and  $15.3 \pm 1.4$   $\mu\text{mol/g}$  muscle respectively. These values were not different statistically from those measured in gastrocnemius muscles of four ARF rats at the end of a perfusion experiment ( $4.2 \pm 0.1$   $\mu\text{mol/g}$  muscle ATP and  $15.0 \pm 1.6$   $\mu\text{mol/g}$  muscle creatine phosphate). This indicated that perfusion did not lead to exhaustion of high-energy phosphate stores.

Table II shows the net rates of protein degradation

TABLE II  
Net Tyrosine Released from Perfused Hindquarters of SO and Acutely Uremic Rats at 24 and 48 h after Surgery

Time after surgery	SO		Acute uremia	
	Basal	Insulin	Basal	Insulin
h	nmol/g/h			
24	52 $\pm$ 8	11 $\pm$ 6	73 $\pm$ 8	57 $\pm$ 9
48	55 $\pm$ 3	20 $\pm$ 4	109 $\pm$ 12*	84 $\pm$ 8†

The results are expressed as mean $\pm$ SEM from seven to nine perfusions of SO and ARF rats. Results obtained after 48 h of ARF were significantly greater than those obtained at 24 h.

\*  $P < 0.01$ .

†  $P < 0.02$ .

calculated as net tyrosine released by the perfused hindquarters of uremic and control rats at 24 and 48 h after surgery. Net tyrosine release in the absence (basal) or presence of insulin was increased significantly ( $P < 0.001$ ) by uremia. In the basal state, net protein degradation was 40% higher after 24 h of uremia and 98% higher after 48 h. Insulin reduced net tyrosine about 20% from hindquarters of uremic rats after both 24 and 48 h. In marked contrast, insulin substantially ( $P < 0.005$ ) suppressed net tyrosine release by hindquarters of SO rats at 24 h (79%) and 48 h (64%) after surgery. This suggests that muscle protein synthesis or degradation or both were markedly abnormal in uremia. To investigate which of the components of muscle protein turnover was responsible for these changes, we measured the rates of protein synthesis and of total protein degradation in incubated epitrochlearis muscles. As shown in Table III, the basal

TABLE I  
Body Weight and Blood Chemistries of Acutely Uremic and SO Rats

	24 h		48 h	
	SO	Acute uremia	SO	Acute uremia
Initial body weight (g)	215 $\pm$ 10	211 $\pm$ 9	219 $\pm$ 9	213 $\pm$ 13
Change in body weight (%)	-13.2 $\pm$ 0.4	-8.1 $\pm$ 0.5*	-19.0 $\pm$ 0.4	-12.1 $\pm$ 0.5*
Serum urea nitrogen (mg/dl)	12 $\pm$ 3	98 $\pm$ 17*	14 $\pm$ 1	157 $\pm$ 12*
Serum creatinine (mg/dl)	0.3 $\pm$ 0.1	4.6 $\pm$ 0.2*	0.5 $\pm$ 0.1	6.4 $\pm$ 0.4
Serum glucose (mg/dl)	132 $\pm$ 10	117 $\pm$ 12	104 $\pm$ 10	135 $\pm$ 12
Serum insulin ( $\mu\text{U/ml}$ )	8.5 $\pm$ 2.2	12.6 $\pm$ 5.4	7.3 $\pm$ 1.2	5.6 $\pm$ 1.0
Arterial blood pH	7.36 $\pm$ 0.05	7.34 $\pm$ 0.02	7.34 $\pm$ 0.03	7.27 $\pm$ 0.02
Plasma sodium (meq/liter)	142 $\pm$ 1	142 $\pm$ 2	143 $\pm$ 3	141 $\pm$ 1
Plasma potassium (meq/liter)	5.0 $\pm$ 0.2	7.4 $\pm$ 0.4*	4.3 $\pm$ 0.2	9.1 $\pm$ 0.5*
Plasma calcium (mg/dl)	8.2 $\pm$ 0.9	9.6 $\pm$ 0.4	9.1 $\pm$ 0.5	8.0 $\pm$ 0.3

Mean $\pm$ SEM values for groups of 6 to 10 rats are shown.

\*  $P < 0.01$  compared with SO rats.

TABLE III  
Components of Protein Turnover in the Incubated Epitrochlearis Muscle of Fasted SO and ARF Rats

	SO			ARF		
	Basal	Insulin	Change	Basal	Insulin	Change
			%			%
Protein synthesis (nmol Phe/g/h)						
24 h	33±3	56±4	+64±4	31±3	51±3	+69±6
48 h	36±1	54±3	+52±5	30±1*	46±1†	+69±9
Protein degradation (nmol Tyr/g/h)						
24 h	142±5	119±9	-23±4	179±7†	161±6*	-10±2
48 h	140±4	112±3	-20±3	169±6*	140±8*	-16±3

The results are expressed as mean±SEM from experiments using at least six rats. The change induced by insulin was significant ( $P < 0.001$ ).

\*  $P < 0.01$ , compared with results from SO rats.

†  $P < 0.02$ , compared with results from SO rats.

rates of muscle protein synthesis in ARF and SO rats after 24 h were comparable, but the basal rate of protein degradation in uremic muscles was significantly higher than in SO muscles ( $P < 0.025$ ). Insulin stimulated protein synthesis similarly in ARF and SO muscles after 24 h. In contrast, insulin was less effective in suppressing protein degradation in ARF, lowering it only 10% while inducing a 23% decrease in SO muscle ( $P < 0.02$ ).

After 48 h, the basal rate of protein synthesis was lower ( $P < 0.005$ ) and the basal rate of protein degradation remained higher ( $P < 0.025$ ) in muscles from ARF rats. At this more advanced stage of ARF, insulin induced a similar percentage increase in the rate of muscle protein synthesis in both ARF and SO muscles. However, the absolute rates of both basal and insulin-stimulated protein synthesis were lower in muscles from ARF rats ( $P < 0.02$ ). At 48 h, muscle protein degradation measured in the presence of insulin was significantly higher in ARF rats compared with SO rats ( $P < 0.01$ ).

Fig. 2 shows the effects of incremental doses of insulin on the rate of protein synthesis in incubated epitrochlearis muscles. This figure indicates that there was resistance to the anabolic effects of insulin after 48 h of ARF (30). At every level of insulin, protein synthesis was higher in muscles of the control rats.

To confirm the development of defective muscle protein synthesis in ARF, the perfused hindquarter was studied. After 48 h, basal protein synthesis ( $26 \pm 3$  ARF vs.  $38 \pm 3$  SO, nmol phenylalanine incorporated/g per h) was 30% lower in perfused muscle from ARF rats (Fig. 3). With insulin, protein synthesis was stimulated to a similar degree in perfused hindquarters of both SO and ARF rats, but once again the absolute

rate ( $54 \pm 5$  ARF vs.  $73 \pm 7$  SO nmol phenylalanine/g per h) was 25% lower in ARF ( $P < 0.05$ ).

Table IV shows the rates of glucose uptake and lactate release by the perfused hindquarters of SO and ARF rats 24 and 48 h after surgery. Basal rates of glucose uptake were similar at 24 h after surgery and similar to rates previously reported for rats starved 48 h (14). In contrast, when insulin was added, the increase in glucose uptake during perfusions of ARF rats

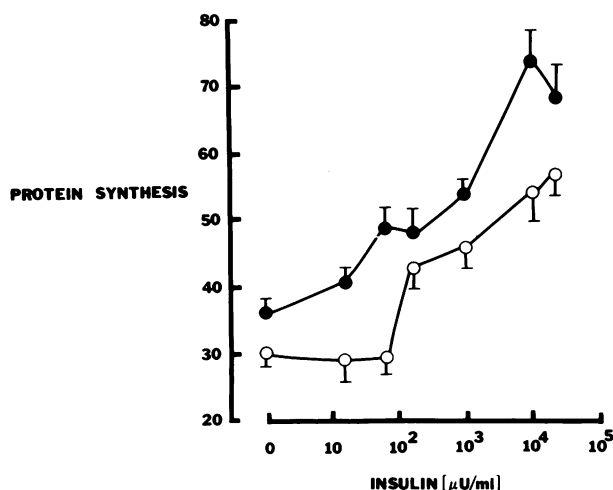


FIGURE 2 The rate of protein synthesis (nanomoles phenylalanine per gram per hour) measured in epitrochlearis muscles when the incubation media contained different concentrations of insulin. Protein synthesis is expressed as nanomoles of phenylalanine incorporated into a gram of muscle per hour of incubation. O, values (mean ± SEM) from ARF rats; ●, values from SO rats. The rates were significantly ( $P < 0.05$ ) different at 0, 15, 75, 1,000, and 10,000  $\mu\text{U/ml}$  insulin.

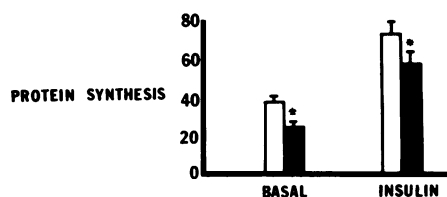


FIGURE 3 The mean ( $\pm$ SEM) rates of muscle protein synthesis measured in the isolated perfused hindquarter from SO (open bars) and ARF (closed bars) at 48 h after surgery are shown. The rates of basal and insulin-stimulated protein synthesis (nanomoles per gram per hour) were lower ( $P < 0.05$ ) in hindquarters of ARF rats.

was 33% less ( $P < 0.001$ ) than that measured in SO rats. At 48 h after surgery, basal glucose uptake by the hindquarters of ARF and SO rats was similar and higher than at 24 h. However, the same pattern was found; the increase in glucose uptake by the hindquarter during stimulation by insulin was 44% less ( $P < 0.02$ ) in ARF rats compared with SO rats.

During perfusion of hindquarters of uremic rats, the basal rate of lactate release at 24 h was  $\sim 25\%$  greater than that of the SO rats, although this difference was not different statistically. By 48 h, the basal rate of lactate release was  $\sim 36\%$  higher in ARF rats compared with SO rats ( $P < 0.005$ ). Thus, as uremia progressed, the basal lactate release increased 43%. This could not be accounted for by fasting alone, since the increase in lactate release measured after 48 h of starvation of SO rats was only 30%. Addition of insulin after 24 and 48 h increased lactate release above basal rates by a comparable percentage in both groups of

rats, but it always was higher in ARF rats (Table IV). Thus, lactate release from peripheral tissues was increased after 24 and 48 h of uremia both in the presence and absence of insulin.

The ratio of lactate release to glucose uptake (L/R) was calculated as an estimate of the efficiency of glucose utilization. In the basal state, it was 18% higher from the hindquarters of ARF rats after 24 h and was 39% higher ( $P < 0.005$ ) after 48 h compared with SO rats. The addition of insulin significantly reduced L/R in SO rats after both 24 (43%;  $P < 0.005$ ) and 48 (38%;  $P < 0.02$ ) h. However, in ARF rats, insulin did not have a significant effect on this ratio.

To examine the other major metabolite released during anaerobic metabolism of glucose, we measured the efflux of alanine from perfused hindquarters after 48 h. Previously, we found that the basal rate of alanine release was  $854 \pm 18$  from ARF and  $736 \pm 24$  nmol/g per h from SO rats (4). In the present experiments, addition of insulin to the perfusate decreased alanine release to  $145 \pm 15$  nmol/g per h during perfusion of SO rats ( $P < 0.005$ ). In contrast, alanine release by hindquarters of ARF rats perfused with insulin was significantly higher ( $557 \pm 26$  nmol/g per h;  $P < 0.005$ ). Thus, in ARF, basal lactate and alanine release were higher and less responsive to the effects of insulin.

To examine the effects of ARF on glycogen synthesis in muscle, we measured the rate of incorporation of glucose into the glycogen of several muscles of the perfused hindquarter (Table V). The basal rate of net glycogen synthesis in ARF was lower in each of the four muscles sampled, although the differences were not significant statistically. When insulin was added,

TABLE IV  
Glucose Uptake and Lactate Release by Perfused Hindquarters of Fasted SO and ARF Rats

	SO		ARF	
	Basal	Insulin	Basal	Insulin
Glucose uptake ( $\mu\text{mol/g/h}$ )				
24 h	$4.61 \pm 0.26$	$13.97 \pm 0.36$	$4.66 \pm 0.30$	$11.02 \pm 0.56^*$
48 h	$6.37 \pm 0.51$	$13.65 \pm 0.73$	$6.84 \pm 0.44$	$10.94 \pm 0.74\dagger$
Lactate release ( $\mu\text{mol/g/h}$ )				
24 h	$3.50 \pm 0.42$	$5.78 \pm 0.64$	$4.37 \pm 0.55$	$7.67 \pm 0.71^*$
48 h	$4.58 \pm 0.35$	$5.33 \pm 0.51$	$6.25 \pm 0.29\dagger$	$7.46 \pm 0.59\dagger$
L/R				
24 h	$0.76 \pm 0.09$	$0.43 \pm 0.03$	$0.90 \pm 0.07$	$0.72 \pm 0.06\dagger$
48 h	$0.66 \pm 0.04$	$0.41 \pm 0.06$	$0.95 \pm 0.08$	$0.75 \pm 0.07\dagger$

The results are expressed as mean  $\pm$  SEM from eight to 12 perfusions of SO and ARF rats. Insulin did not suppress the L/R significantly after 24 h of ARF but altered it slightly at 48 h ( $P < 0.05$ ). In contrast, insulin effectively suppressed the ratio at 24 ( $P < 0.005$ ) and 48 ( $P < 0.02$ ) h in SO rats.

\*  $P < 0.05$ , compared with results obtained during perfusion of SO rats.

†  $P < 0.01$ , compared with results obtained during perfusion of SO rats.

TABLE V  
Net Rate of Muscle Glycogen Synthesis in Perfused Hindquarters of ARF and SO Rats after 48 h

Rats	Soleus		Red gastrocnemius		White gastrocnemius		Extensor digitorum longus	
	Basal	Insulin	Basal	Insulin	Basal	Insulin	Basal	Insulin
$\mu\text{mol/g wet wt/h}$								
ARF	0.68±0.26	7.89±1.45	0.35±0.21	7.65±0.85*	0.24±0.08	1.55±0.38*	0.23±0.08†	6.26±0.70*
SO	1.89±0.86	10.78±1.06	1.49±0.72	13.96±1.64	0.53±0.28	3.63±0.50	1.41±0.43	13.72±1.04

The results (mean±SEM) of four paired perfusions in the absence and of eight perfusions in the presence (plus insulin) of 0.05 mU/ml insulin are presented.

\*  $P < 0.01$ , compared with muscles from SO rats.

†  $P < 0.05$ , compared with muscles from SO rats.

glucose flux into glycogen was significantly lower in all but the slow-twitch, red soleus muscle of ARF rats.

The observations that ARF caused the concurrent development of partial resistance both to insulin-mediated glucose metabolism and to insulin-mediated suppression of net protein degradation suggested that these defects might be related. Therefore, the rate of net tyrosine release was compared with the lactate release/glucose uptake ratio in individual experiments performed with and without insulin at 48 h after surgery. As shown in Fig. 4, there was a significant positive correlation ( $r = 0.78$ ;  $P < 0.001$ ) such that as the lactate release/glucose uptake ratio increased there was a higher net rate of tyrosine released.

## DISCUSSION

These results document that ARF induces abnormal muscle protein turnover and that the abnormalities become more pronounced as uremia becomes more severe. Moreover, we found that the ability of insulin

to correct changes in net protein turnover is reduced by ARF. After 24 h of ARF, the increase in net tyrosine release from the hindquarter was due primarily to increased muscle protein degradation, which responded poorly to insulin. In contrast, the basal rates of muscle protein synthesis in ARF and SO rats were comparable, and they were stimulated to a similar degree by insulin. Others (31), using a cell-free preparation with ribosomes isolated from rat muscle, have also reported normal rates of protein synthesis 24 h after nephrectomy. After 48 h of ARF, we found that protein synthesis in both incubated and perfused muscle was depressed in addition to the augmented rate of protein degradation (Table III, Fig. 3). Clearly, these changes in muscle protein turnover could account for the increased release of amino acids from the hindquarters of acutely uremic rats we noted previously (4).

These experiments confirm that there is resistance to insulin-stimulated glucose uptake by peripheral tissues early in ARF (5). They also show that this defect

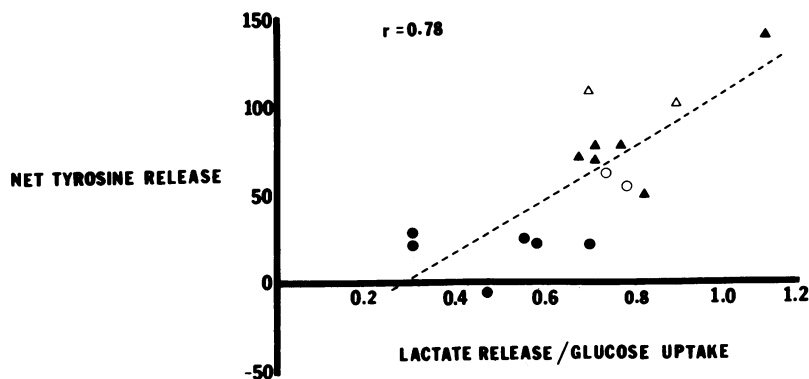


FIGURE 4 The relationship between net tyrosine release (nanomoles per gram per hour) and L/R by the perfused hindquarter in individual experiments 48 h after surgery is shown. ▲ and ● represent values from ARF and SO rats, respectively, perfused in the presence of insulin; △ and ○ represent values from ARF and SO rats, respectively, perfused without added insulin. The relationship is significant ( $r = +0.78$ ;  $P < 0.001$ ).

persists through 48 h of ARF and that there are abnormalities in glucose disposal. The possible fates of glucose taken up by muscle include entry into glycogen or anaerobic metabolism to pyruvate, which can enter the Krebs cycle for oxidative metabolism or can be converted to lactate or alanine for release into the circulation (32). This presumes that futile cycling of glucose, entry into the pentose phosphate shunt, and the intracellular accumulation of glycolytic metabolites are minimal in muscle from fasted rats (33–36). After 48 h, we found that in ARF the basal rate of lactate released could account for ~47% of the glucose taken up and that insulin did not change this proportion (38%) significantly. In contrast, the basal efflux of lactate from control hindquarters accounted for only 33% of the glucose taken up and was reduced to 20% ( $P < 0.05$ ) by insulin. Since alanine release also was suppressed to a greater extent by insulin in SO compared with ARF rats, it must be concluded that the ability of insulin to direct the disposal of glucose into more preferable metabolic pathways such as oxidative metabolism or glycogen synthesis (32) was reduced. Since oxidative metabolism represents <5% of total glucose disposal by the perfused hindquarter of fasted rats (36), it would appear most likely that a block in glycogen deposition develops in acute uremia. This conclusion is supported by the results in Table V. Net glycogen synthesis clearly was lower in muscles of ARF compared with SO rats. Thus, after 24 h of ARF, three defects in insulin-responsive muscle metabolism were observed: there was a reduced effect of insulin on proteolysis, insulin-induced glucose uptake was diminished, and the ability of insulin to reduce the proportional release of lactate was decreased. These persisted through 48 h of acute uremia.

After 48 h of ARF, in both the perfused hindquarter (Fig. 3) and incubated muscle (Fig. 2, Table III), basal and insulin-stimulated muscle protein synthesis were reduced. Since insulin was able to increase protein synthesis in ARF and in SO muscle by a similar percentage, the sensitivity to insulin apparently was not impaired. In fact, no significant difference in the half-maximal stimulation of protein synthesis in muscles from SO and ARF rats could be detected (Fig. 2). On the other hand, the rate of protein synthesis in uremic muscles was lower at each level of insulin tested, indicating clearly the presence of insulin resistance (30). Although starvation can reduce the capacity for protein synthesis (11), the present results indicate that ARF induces an additional, presumably postreceptor, defect in insulin-stimulated muscle protein synthesis. The appearance of this additional defect presumably accounts for the further increase in net tyrosine release by the perfused hindquarters of ARF rats after 48 h, compared with that occurring after 24 h (Table II).

There have been few reported studies examining the components of muscle protein turnover in rats with ARF. 48 h after bilateral nephrectomy, Shear (37) injected [ $^{14}\text{C}$ ]leucine into rats and found that incorporation of the label into muscle protein was reduced; rates of muscle protein catabolism and net protein turnover were not reported. Differences in the rates of uptake and of metabolism of leucine also were not reported. Since alterations in either of these could affect the intracellular specific radioactivity of leucine and hence the rate of incorporation of the label into muscle protein (18), it is unclear whether this finding was due solely to decreased protein synthesis.

Apparently, muscle protein degradation is increased in immature (90 g) rats after 24 h of ARF, while muscle protein synthesis is unaffected (38). In that preliminary report, since the effects of insulin and 48 h of ARF on rates of muscle protein synthesis and degradation were not included, it is unknown whether immature rats with ARF also develop progressive defects in muscle protein turnover. Flugel-Link et al. (39), using the hemicorpus perfusion technique, found that in the absence of insulin the rate of muscle protein synthesis of starved, adult rats 30 h after bilateral nephrectomy was ~33% lower than in SO rats, but the difference was not significant statistically. On the other hand, net protein degradation was increased significantly by ~37%. From these reports (38, 39) and the present data, the initial abnormality in muscle protein turnover induced by ARF appears to be accelerated protein degradation.

The concurrent appearance of defects in the responsiveness of glucose metabolism and muscle protein turnover to insulin in ARF suggests that abnormal regulation of muscle protein turnover may be a consequence of defective glucose utilization. This is supported by the relationship shown in Fig. 4, in which inefficient glucose utilization was found to be correlated with increased net protein degradation. First, net protein degradation may have increased because energy extraction from glucose was restricted, necessitating energy extraction from amino acids (and especially branched-chain amino acids; 40). Another potential explanation would be that a change in the redox state of muscle caused a secondary change in the rate of net protein degradation. Tischler (41) and Heddon and Buse (42) have suggested that a decrease in the cytoplasmic NADH/NAD ratio is associated with an increase in the net rate of muscle protein degradation. In the present study, the increased proportional efflux of lactate from muscle compared with the uptake of glucose could have lowered the NADH/NAD ratio and, according to this hypothesis, could have caused a net increase in proteolysis.

Clearly, the correlation we found (Fig. 4) does not



necessarily mean that defective glucose metabolism caused an increase in net protein degradation. It is possible that the two defects developed in parallel and that some other mechanism, e.g., amino acid deficiency caused by defective amino acid transport (6), the accumulation of a uremic toxin (43), or resistance to the direct inhibitory effect of insulin on muscle protein degradation, led to increased protein degradation. Nevertheless, the close relationship between inefficient glucose utilization and impaired protein metabolism plus the temporally associated, progressive development of these abnormalities in ARF suggest that all may be linked to a single metabolic defect.

## ACKNOWLEDGMENTS

We wish to thank M. Adam and L. Perry for technical assistance, Dr. J. Warram for his help with the biostatistical analysis, Dr. T. Aoki for measuring serum insulin concentrations, and P. Dolan for expert editorial assistance.

This work was supported by grants AM 26903, a National Research Service Award, AM 06660, to Dr. Clark and a Research Career Development Award, AM 00750, to Dr. Mitch from the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, National Institutes of Health.

## REFERENCES

1. Lacy, W. E. 1969. Effect of acute uremia on amino acid uptake and urea production by perfused rat liver. *Am. J. Physiol.* 216:1300-1305.
2. Fröhlich, J., J. Scholmerich, G. Hoppe-Seyler, K. P. Maier, H. Talke, P. Schollmeyer, and W. Gerok. 1974. The effect of acute uremia on gluconeogenesis in isolated perfused rat livers. *Eur. J. Clin. Invest.* 4:453-458.
3. Bondy, P. K., F. L. Engel, and B. Farrar. 1949. The metabolism of amino acids and protein in the adrenalectomized-nephrectomized rat. *Endocrinology*. 44:476-483.
4. Mitch, W. E. 1981. Amino acid release from the hindquarter and urea appearance in acute uremia. *Am. J. Physiol.* 241:E415-E419.
5. Mondon, C. E., C. B. Dolkas, and G. M. Reaven. 1978. The site of insulin resistance in acute uremia. *Diabetes*. 27:571-576.
6. Arnold, W. E., and M. A. Holliday. 1979. Tissue resistance to insulin stimulation of amino acid uptake in acutely uremic rats. *Kidney Int.* 16:124-129.
7. Jefferson, L. S., J. B. Li, and S. R. Rannels. 1977. Regulation by insulin of amino acid release and protein turnover in the perfused rat hemi-corpus. *J. Biol. Chem.* 252:1476-1483.
8. Garber, A. J. 1978. Skeletal muscle protein and amino acid metabolism in experimental chronic uremia in the rat. Accelerated alanine and glutamine formation and release. *J. Clin. Invest.* 62:623-632.
9. Preedy, V. R., and P. J. Garlick. 1981. Rates of protein synthesis in skin and bone, and their importance in the assessment of protein degradation in the perfused rat hemi-corpus. *Biochem. J.* 194:373-376.
10. Li, J. B., and A. L. Goldberg. 1976. Effects of food deprivation on protein synthesis and degradation in rat skeletal muscles. *Am. J. Physiol.* 231:441-448.
11. Li, J. B., J. E. Higgins, and L. S. Jefferson. 1979. Changes in protein turnover in skeletal muscle in response to fasting. *Am. J. Physiol.* 236:E222-E228.
12. Goodman, M. N., M. A. McElaney, and N. B. Ruderman. 1981. Adaptation to prolonged starvation in the rat: curtailment of skeletal muscle proteolysis. *Am. J. Physiol.* 241:E321-E327.
13. Mitch, W. E., and W. Chan. 1979.  $\alpha$ -Ketoisocaproate stimulates branched-chain amino acid transaminase in kidney and muscle. *Am. J. Physiol.* 236:E514-E518.
14. Ruderman, N. B., C. R. S. Houghton, and R. Hems. 1971. Evaluation of the isolated perfused rat hindquarter for the study of muscle metabolism. *Biochem. J.* 124:639-651.
15. Wang, M., I. Vyhmeister, J. D. Kopple, and M. E. Swendseid. 1976. Effect of protein intake on weight gain and plasma amino acid levels in uremic rats. *Am. J. Physiol.* 230:1455-1459.
16. Richter, E. A., L. P. Garetto, M. N. Goodman, and N. B. Ruderman. 1982. Muscle glucose metabolism following exercise in the rat. Increased sensitivity to insulin. *J. Clin. Invest.* 69:785-793.
17. Nesher, R., I. E. Karl, K. E. Kaiser, and D. M. Kipnis. 1980. Epitrochlearis muscle. I. Mechanical performance, energetics, and fiber composition. *Am. J. Physiol.* 239:E454-E460.
18. Li, J. B., R. M. Fuls, and A. L. Goldberg. 1973. Evidence that the intracellular pool of tyrosine serves as precursor for protein synthesis in muscle. *J. Biol. Chem.* 248:7272-7275.
19. Clark, A. S., and W. E. Mitch. 1983. Comparison of protein synthesis and degradation in incubated and perfused muscle. *Biochem. J.* 212:649-653.
20. Brady, L. J., M. N. Goodman, F. N. Kalish, and N. B. Ruderman. 1981. Insulin binding and sensitivity in rat skeletal muscle: effect of starvation. *Am. J. Physiol.* 240:E184-E190.
21. Andrews, T. M., R. Goldthorp, and R. E. Watts. 1973. Fluorimetric measurements of the phenylalanine content of human granulocytes. *Clin. Chim. Acta.* 43:379-387.
22. Faulkner, W. R. 1965. Phenylalanine. *Stand. Methods Clin. Chem.* 5:199-209.
23. Waalkes, T. P., and S. Udenfried. 1957. A fluorimetric method for the estimation of tyrosine in plasma and tissues. *J. Lab. Clin. Med.* 50:733-736.
24. Huggett, A., and D. A. Nixon. 1957. Use of glucose oxidase, peroxidase, and o-dianisidine in determination of blood and urinary glucose. *Lancet*. II:368-370.
25. Good, C. A., H. Kramer, and M. Somogyi. 1933. The determination of glycogen. *J. Biol. Chem.* 100:485-491.
26. Fawaz, G., and E. N. Fawaz. 1971. Phosphate compound analyses. *Methods Pharmacol.* 1:515-551.
27. Soeldner, J. S., and D. Sloane. 1965. Critical variables in the radioimmunoassay of serum insulin using the double antibody technique. *Diabetes*. 14:771-779.
28. Kuzuya, H., P. M. Blix, D. L. Horwitz, D. F. Steiner, and A. H. Rubenstein. 1977. Determination of free and total insulin and C-peptide in insulin-treated diabetics. *Diabetes*. 26:22-29.
29. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames. Sixth ed. 258-334.
30. Kahn, C. R. 1978. Insulin resistance, insulin sensitivity, and insulin unresponsiveness: A necessary distinction. *Clin. Endocrinol. Metab.* 27(Suppl. 2):1893-1902.

31. Pils, P., W. Jettmar, D. Adamiker, and K. H. Tragl. 1981. Insulin and the in vitro protein synthesis of liver and skeletal muscle ribosomes in experimental acute uremia. *Horm. Metab. Res.* 13:89-91.
32. Ruderman, N. B., M. N. Goodman, C. A. Conover, and M. Berger. 1979. Substrate utilization in perfused skeletal muscle. *Diabetes*. 28:13-17.
33. Hae, L., and H. G. Hers. 1974. On the use of ( $^3\text{H}$ ,  $^{14}\text{C}$ ) labelled glucose in the study of the so-called "futile" cycle in liver and muscle. *Biochem. Biophys. Res. Comm.* 58:432-439.
34. Nesher, R., I. E. Karl, and D. M. Kipnis. 1980. Epitrochlearis muscle. II. Metabolic effects of contraction and catecholamines. *Am. J. Physiol.* 239:E461-E467.
35. Katz, J., and R. Rognstad. 1976. Futile cycles in the metabolism of glucose. *Curr. Top. Cell. Regul.* 10:237-289.
36. Berger, M., S. A. Hagg, M. N. Goodman, and N. B. Ruderman. 1976. Glucose metabolism in perfused skeletal muscle. Effects of starvation, diabetes, fatty acids, acetoacetate, insulin and exercise on glucose uptake and disposition. *Biochem. J.* 158:191-202.
37. Shear, L. 1969. Internal redistribution of tissue protein synthesis in uremia. *J. Clin. Invest.* 48:1252-1257.
38. Kunin, A. S. 1982. Effect of acute renal failure on muscle protein turnover. *Clin. Res.* 30:245A. (Abstr.)
39. Flugel-Link, R. M., I. B. Salusky, M. R. Jones, and J. D. Kopple. 1982. Enhanced muscle protein degradation and urea nitrogen appearance in rats with acute renal failure. *Kidney Int.* 21:217. (Abstr.)
40. Goldberg, A. L., and T. W. Chang. 1978. Regulation and significance of amino acid metabolism in skeletal muscle. *Fed. Proc.* 37:2301-2307.
41. Tischler, M. E. 1980. Is regulation of proteolysis associated with redox-state changes in rat skeletal muscle? *Biochem. J.* 192:963-966.
42. Hedden, M. P., and M. G. Buse. 1982. Effects of glucose, pyruvate, lactate, and amino acids on muscle protein synthesis. *Am. J. Physiol.* 242:E184-E192.
43. Cernacek, P., V. Spustova, and R. Dzurik. 1982. Inhibitor(s) of protein synthesis in uremic serum and urine partial purification and relationship to amino acid transport. *Biochem. Med.* 27:305-316.