PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 169, 519–526 (1982)

Isolation of Protein–Glycogen Complexes from Rat Skeletal Muscle in Acute Uremia: Role of Serine (41383)

ROLAND M. SCHÄFER, WALTER H. HÖRL, AND AUGUST HEIDLAND

Department of Medicine, Division of Nephrology, University of Würzburg, 8700 Würzburg, West Germany

Abstract. Attempts were made to isolate an intact protein–glycogen complex from rat skeletal muscle in acute and chronic uremia and after different treatment regimes. In control animals the amount of glycogen in the 30-pellet fraction was 16.5 mg and in rats with chronic uremia 27.6 mg. The yields of glycogen phosphorylase and synthetase do not correlate with the quantity of glycogen during isolation. In the presence of ATP, calcium, and magnesium, flash activation of phosphorylase was four to six times slower in rat than in rabbit muscle. In acute uremia, after feeding a mixed low-protein diet and Walser ketoacid mixture, a protein–glycogen complex could not be isolated. Additional administration of propranolol resulted in yields of only 10 and 7 μg glycogen after binephrectomy and ureter ligation, respectively. In contrast, glycogen concentration increased dramatically after sham operation (4.55 mg glycogen/mg protein). Additional administration of high doses of serine resulted in an increase of glycogen in the complex of 21.5 mg (134.3 μg glycogen/mg protein) in acute uremia. Kinetic analysis of phosphorylase activation in the presence of ATP, calcium, and magnesium indicated an intact protein–glycogen complex under these conditions. Glycogen concentrations were correlated with phosphorylase phosphatase activity. Constant amounts of sarcoplasmic reticulum calcium ATPase were isolated under all conditions tested. Our data suggest a key role of serine in the glycogen metabolism of muscle in acute uremia.

It has long been established that under uremic conditions, glycogen metabolism of striated muscle is altered. However, the changes which take place in chronic uremia greatly differ from those present during acute renal insufficiency. Bergström and Hultman (1) found a distinct fall in muscle glycogen of patients with acute renal failure, whereas glycogen content was normal in patients with chronic renal insufficiency. The following factors may contribute to the altered muscle glycogen metabolism in uremia: insulin resistance (2), catecholamine stimulation of glycogenolysis (3, 4), calcium activation of phosphorylase kinase through alterations in the calcium transport system of the sarcoplasmic reticulum (4, 5), proteolytic activation of the key enzyme of glycogen metabolism (6, 7), and uncontrolled actomyosin ATPase by altered subunit structure of the troponin complex (3).

A study of the protein–glycogen complex was undertaken to provide further information on the regulation of glycogenolysis in different experimental models of acute and chronic uremia. This structure contains all enzymes which are involved in the regulation of muscle glycogen metabolism (8). The Ca^{2+} activation of phosphorylase *b* (EC 2.4.1.1) and phosphorylase kinase (EC 2.7.1.38) is associated with the muscle protein–glycogen complex in a way similar to that seen in intact tissue during muscle contraction (9). In parallel with kinase activation the antagonistic enzyme phosphorylase phosphatase (EC 3.1.3.17) is inhibited in the muscle protein–glycogen complex (10), whereas no such regulation could be observed on purified enzymes. Furthermore, phosphorylase *b* can be allosterically activated by AMP. In resting muscle phosphorylase *b* is essentially inactive in spite of the presence of 0.4 m*M* AMP, 7.2 m*M* ATP, and 0.2 m*M* glucose-6-*P* (11). Purified phosphorylase *b* dissolved in the same medium exhibits approximately 100 times more activity (12). Attention was therefore focused on the protein–glycogen complex.

The aims of the present study were:

0037-9727/82/040519-08$01.00/0

(1) to isolate and characterize protein–glycogen complexes from rat skeletal muscle in different states of uremia;

(2) to examine the possible effects of a high-calorie mixed low-protein diet in combination with the following:

(a) selected amino acids and their keto analogs to examine their effects on glycogen synthesis and to assess the nitrogen-sparing effects of the keto analogs;

(b) propranolol to abolish the catecholamine-induced stimulation of glycogenolysis in acute uremia;

(c) high doses of serine since this amino acid was shown to stimulate gluconeogenesis in isolated hepatocytes (7) as well as muscle glycogen synthesis (4) in different models of acute uremia.

Materials and Methods. *1. Experimental protocol.* Female Sprague–Dawley rats (Fa. Wiga, Sulzfeld, West Germany) with an initial weight of 180–220 g were used for the experiment. Animals were separately housed in individual cages in a room at constant temperature and humidity with a controlled light cycle of 12 hr on and 12 hr off. Until the time of the experiment, the animals had access to water and Altromin standard feed *ad libitum*. All the operations were performed under ether anesthesia. Untreated control animals (CO) and chronically (90% nephrectomy) uremic rats (CU) had free access to food and water. Sham-operated (SOP), binephrectomized (BN), and ureter-ligated (UL) rats were postoperatively caged individually and tube fed every 8 hr as follows:

(a) on a mixed low-protein diet (MLPD, Berodiät V, produced by Boehringer, Ingelheim, West Germany, containing: 33.4 g carbohydrate, 11.4 g fat, 0.7 g protein, 1.7 mg vitamin E – acetate = a total of 48 g) in a dosage of approximately 40 kcal/kg/24 hr at a protein ration of 0.12 g/kg;

(b) on a MLPD in combination with the mixture of essential amino acids and keto analogs (KAA according to Walser (24))—this mixture was prepared by Pfrimmer & Company, Erlangen, West Germany;

(c) on a MLPD, KAA in combination with the β blocking agent (BB) propranolol (Dociton; ICI-Pharma, Plankstadt, West Germany, 0.8 mg/kg/8 hr subcutaneously);

(d) on a MLPD, KAA in combination with propranolol and a high dose of *l*-serine (0.5 g/kg/8 hr); *l*-serine (SER) was obtained from Pfrimmer & Company, Erlangen, West Germany.

Animals were killed by decapitation 60 hr after the induction of acute uremia and 8 weeks after the induction of chronic uremia. Isolation of the protein–glycogen complex was carried out according to Meyer *et al.* (8) using the first of the three procedures they describe. This group isolated a protein–glycogen complex from rabbit skeletal muscle. In the present study, when rat skeletal muscle was used, it was necessary to remove muscle from 8 to 10 animals for each study group, in order to obtain sufficient muscle (minimum 100 g) for protein–glycogen complex analysis. Each animal was sacrificed and immediately placed in iced water for 15 min in order to prevent muscle glycogen degradation. Each of the experiments was performed in duplicate (180 rats).

2. Analytical methods and measurements. The material obtained by acid precipitation of the crude extract (CE) and following differential centrifugation (90 min at 80,000*g*, i.e., 30,000 rpm) will be referred to as the 30-pellet fraction (30-P). The flash activation of phosphorylase in the glycogen particle was performed according to the method of Heilmeyer *et al.* (9). Protein was anlayzed by the method of Lowry *et al.* (13); glycogen was estimated by a modification of the Montgomery method (14) as previously described (15); phosphate was determined by the method of Fiske and Subbarow (16); phosphorylase activity was estimated according to Hedrick and Fischer (17), and glycogen synthetase (EC 2.4.1.11) by the method of Thomas *et al.* (18). Phosphorylase kinase was estimated by the method of Jennissen and Heilmeyer (19). This enzyme was isolated according to the method of Cohen (20) with the modification described by Jennissen and Heilmeyer (21). Phosphorylase *b* crystallized three times was prepared using the method of Krebs and Fischer (22) and phosphorylase phosphatase activity was measured by the

method of Haschke *et al.* (10). The activity of the calcium transport ATPase (EC 3.6.1.3) in the sarcoplasmic reticulum was measured according to the method of De-Meis and Hasselbach (23) with the modifications of Hörl *et al.* (24). γ^{32}P-Labeled ATP was prepared by the method of Glynn and Chappel (25). Gel electrophoresis in the presence of sodium dodecyl sulfate was carried out according to the method of Weber and Osborn (26). Blood urea nitrogen (BUN) was analyzed automatically by routine methods.

Results. Protein–glycogen complexes were isolated from skeletal muscle of:

(I) untreated control rats (BUN 16.5 ± 1.2 mg/dl),

(II) chronically uremic rats (BUN 78.3 ± 6.1 mg/dl),

(III) sham-operated rats tube fed with MLPD and KAA

(IV) sham-operated rats fed with MLPD, KAA, and injected with propranolol,

(V) bilaterally nephrectomized rats fed with MLPD and KAA (BUN 380 ± 16 mg/dl),

(VI) bilaterally nephrectomized rats fed with MLPD, KAA, and injected with propranolol (BUN 362 ± 16 mg/dl),

(VII) ureter-ligated rats fed with MLPD, KAA, and injected with propranolol (BUN 363 ± 18 mg/dl),

(VIII) bilaterally nephrectomized rats fed with MLPD, KAA, SER, and injected with propranolol (BUN 340 ± 14 mg/dl),

(IX) bilaterally nephrectomized rats fed with MLPD, KAA in a high dosage of 2.1 g/kg/24 hr (10-fold higher than in groups III–VIII) and injected with propranolol (BUN 355 ± 12 mg/dl),

(X) bilaterally nephrectomized rats fed with MLPD, SER, and injected with propranolol (BUN 365 ± 21 mg/dl).

Animals of groups III–X were decapitated 60 hr after operation. The levels of glycogen, glycogen phosphorylase, and synthetase activities are shown in Tables I–III. It can be seen that injection of propranolol results in high levels of glycogen in the 30-pellet fraction of sham-operated rats supplemented with high-calorie, low-protein diet and ketoacids according to Walser. This could not be seen in rats 60 hr after bilateral nephrectomy or ureter ligation under these conditions (groups VI and VII). However, after the additional administration of high doses of serine high levels of glycogen could be measured in crude muscle extracts as well as in protein–glycogen complexes of acutely uremic animals (group VIII).

A strong correlation between glycogen concentration in the protein–glycogen complex and the specific activity of phosphorylase phosphatase was observed. For example, the glycogen concentration in the protein–glycogen complex of untreated control rats was 166.7 μg/mg protein with a phosphorylase phosphatase specific activity of 10.6×10^{-3} unit/mg whereas 60 hr after binephrectomy in animals on a high-calorie diet, the Walser ketoacid mixture and propranolol, the glycogen concentration was 0.11 μg/mg protein in the complex and the specific activity of the enzyme was 0.75×10^{-3} unit/mg. In contrast, the antagonistic enzyme, phosphorylase kinase, seems to be independent of the glycogen concentration in the protein–glycogen complex. For example, in untreated

TABLE I. EFFECTS OF DIFFERENT DIETS ON GLYCOGEN CONTENT (Σ μg) DURING ISOLATION OF THE PROTEIN–GLYCOGEN COMPLEX

Fraction	I	II	III	IV	V	VI	VII	VIII	IX	X
Crude extract	35,400	38,100	1100	25,500	2300	1925	1650	26,300	3050	2250
Acid pellet	17,300	27,850	74	4,830	—	20	20	22,200	1680	100
30-pellet	16,500	27,650	10	4,550	—	10	7	21,500	1545	40
No. of animals	8	8	8	8	10	10	10	10	8	10

Note. Isolation of the protein–glycogen complex (30-pellet) was carried out according to Meyer *et al.* (8). Glycogen was estimated by a modification of the Montgomery method (14). Symbols I–X represent the different treatment regimes as described under Results.

TABLE II. EFFECTS OF DIFFERENT DIETS ON PHOSPHORYLASE ACTIVITY (Σ UNITS) DURING ISOLATION OF THE PROTEIN–GLYCOGEN COMPLEX

Fraction	I	II	III	IV	V	VI	VII	VIII	IX	X
Crude extract	43,900	40,800	38,400	19,550	7800	7300	11,300	10,250	18,700	30,300
Acid pellet	29,600	6,400	4,750	5,100	450	540	580	2,450	2,400	5,800
30-pellet	1,600	1,950	2,010	1,540	230	230	310	2,100	1,450	1,200
No. of animals	8	8	8	8	10	10	10	10	8	10

Note. Isolation of the protein–glycogen complex (30-pellet) was carried out according to Meyer *et al.* (8). Phosphorylase activity was measured according to Hedrick *et al.* (17). Symbols I–X represent the different treatment regimes as described under Results.

controls the specific activity of phosphorylase kinase was 0.25 unit/mg (glycogen 166.7 μg/mg protein) whereas in chronic uremic rats, glycogen concentration was 158.0 μg/mg and the specific activity of the enzyme 0.025 unit/mg. After serine administration during acute uremia when glycogen concentration was 134.4 μg/mg protein, the kinase activity was 0.053 unit/mg protein.

Figure 1 demonstrates the flash activation of phosphorylase in a protein–glycogen complex isolated from untreated rabbits and rats as well as acutely uremic rats. At starting point 0, a small transformation from phosphorylase *b* to *a* takes place (probably due to traces of free calcium ions in the incubation medium). Eight percent phosphorylase in active *a* form could be determined in the protein–glycogen complex of untreated control rabbits and rats. After the addition of ATP, calcium, and magnesium, the enzyme is flash activated. About 60% of the enzyme transforms into active *a* form. The following step of the phosphorylase phosphatase activation causes a dephosphorylation of phosphorylase *a*. Repeated supplements of ATP cause repeated flash activations of phosphorylase. When ATP is added to a protein–glycogen complex obtained from acutely uremic rats treated with a mixed low-protein diet, the Walser ketoacid mixture, propranolol, and high doses of serine, again a small flash activation is triggered by traces of calcium in the medium. After about 1½ min, approximately 30% of the phosphorylase in the active *a* form is present under these conditions. The supplementation of ATP, magnesium, and calcium causes a flash activation of the phosphorylase with a transformation from *b* to *a* of about 68%. Repeated addition of ATP resulted in repeated flash activations of phosphorylase. On the other hand, after the addition of ATP and magnesium to phosphorylase in the 30-pellet suspension obtained from acutely uremic rats exclusively fed on a mixed low-protein diet in combination with the Walser ketoacid mixture, 70% of the enzyme exists in active *a* form. The addition of more calcium and magnesium does not result in further stimulation. Finally, Fig. 1 shows differences of a phosphorylase flash activation in a protein–glycogen complex isolated from

TABLE III. EFFECTS OF DIFFERENT DIETS ON SYNTHETASE ACTIVITY (Σ UNITS) DURING ISOLATION OF THE PROTEIN–GLYCOGEN COMPLEX

Fraction	I	II	III	IV	V	VI	VII	VIII	IX	X
Crude extract	43,000	30,350	15,000	12,120	6420	6500	6700	14,000	10,700	20,200
Acid pellet	16,200	16,500	7,600	5,360	1330	1500	1550	6,050	7,000	9,800
30-pellet	2,850	3,400	2,650	3,000	385	250	550	3,300	2,400	2,200
No. of animals	8	8	8	8	10	10	10	10	8	10

Note. Isolation of the protein–glycogen complex (30-pellet) was carried out according to Meyer *et al.* (8). Synthetase was estimated by the method of Thomas *et al.* (18). Symbols I–X represent the different treatment regimes as described under Results.

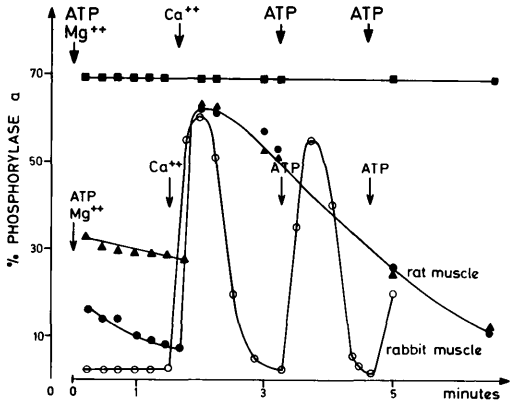

FIG. 1. Differences in flash activation of phosphorylase in the protein–glycogen complex isolated from rat and rabbit skeletal muscle as well as effects of various diets on flash activation of phosphorylase in the glycogen–protein complex isolated from skeletal muscle of acute uremic rats. 460 μl 30-P fraction (25–40 mg protein/ml in 50 m*M* Na-glycerophosphate, 1 m*M* EDTA, pH 6.8) was incubated with 25 μl $MgCl_2$ (100 m*M*) and 10 μl ATP (50 m*M*, pH 7.0). Samples (20 μl) were taken at the times indicated in the figure. 10 μl $CaCl_2$ (40 m*M*) was added after 105 sec. ATP was added as shown in the figure. ○, Untreated rabbits; (I) ●, untreated control rats; (VI) ■, bilaterally nephrectomized rats fed with MLPD, KAA, and injected with propranolol (the specific activities of the enzyme in the presence and absence of AMP (2 m*M*) were 2.61 and 1.07 μmol × min^{-1} × mg^{-1} protein in the 30-P fraction, respectively); (VIII) ▲, bilaterally nephrectomized rats fed with MLPD, KAA, SER, and injected with propranol.

the skeletal muscle of untreated control rats in comparison to a protein–glycogen complex isolated from skeletal muscle of untreated rabbits. It can be seen that after the addition of ATP, calcium, and magnesium, the flash activation and inactivation of phosphorylase in the rabbit muscle protein–glycogen complex proceeds about four times faster.

Figure 2 shows the effects of various ATP concentrations on the conversion of phosphorylase *b* to *a*. At a concentration of 1 mmol/liter ATP, there is an increase to a level of 60% of the *a* form; this decreases to the initial value (approximately 8% *a* form) after 5 min. The identical activation curve is achieved with a concentration of 2.0 mmol/liter ATP. Using an ATP concentration of 0.5 mmol/liter, only a 30% conversion of phosphorylase can be produced; there is a return to initial values after 2 min. Once again, the phosphorylase in the 30-pellet suspension of acutely uremic rats fed on a high-calorie, low-protein diet in combination with the Walser ketoacid mixture is maximally activated and proves to be unaffected by ATP stimulation.

The specific activity of the sarcoplasmic reticulum calcium ATPase was 0.99 μmol × min^{-1} mg $protein^{-1}$ in untreated controls and 0.57 μmol × min^{-1} × mg^{-1} protein in chronic uremia. The specific activity of this enzyme was 0.76 in group IV compared to 1.67 and 1.83 μmol × min^{-1} × mg^{-1} protein in the acutely uremic groups VI and VII, respectively. The yield of the calcium ATPase in the 30-pellet fraction was in the range of 13 to 22% of crude extract values.

Discussion. According to the studies of Meyer *et al.* (8), the protein–glycogen complex isolated from rabbit skeletal muscle is a specific functional cell unit. We were able to isolate and characterize protein–glycogen complexes from rat skeletal muscle in different states of uremia. In contrast to rabbit muscle (8), the percentage yield of phosphorylase during isolation of the protein–glycogen complex under control conditions does not correlate with the glycogen concentration. Obviously, rat muscle glycogen phosphorylase and synthetase exhibit weaker binding to glycogen, than do rabbit muscle glycogen metaboliz-

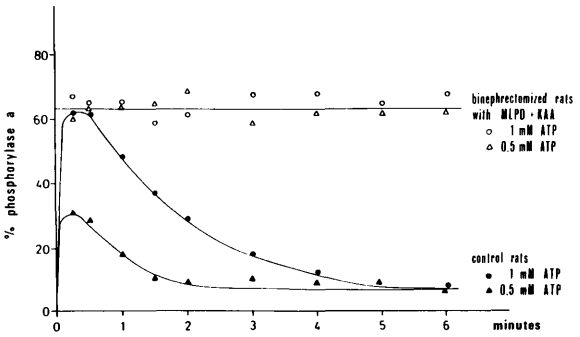

FIG. 2. Effects of different ATP concentrations on phosphorylase *b* to *a* conversion in a protein–glycogen complex. Incubation medium consisted of protein–glycogen complex fraction, 1 and 0.5 mmol/liter ATP, 5 mmol/liter $MgCl_2$ and 1 mmol/liter $CaCl_2$.

ing enzymes. At any rate, there is almost double the yield of phosphorylase *a* compared to *b* during the individual isolation phases. In our opinion, there are two ways of interpreting this phenomenon: either the phosphorylated form of phosphorylase (phosphorylase *a*) might be more tightly bound to glycogen than the unphosphorylated *b* form, or perhaps during isolation, the AMP activation of phosphorylase *b* is partially lost. Hickenbottom (27) has demonstrated the same relationship for glycogen and glycogen synthetase in rabbit skeletal muscle as Meyer *et al.* (8) for glycogen and phosphorylase. In acute uremia, this correlation is valid after isolation of a protein–glycogen complex originating from skeletal muscle in binephrectomized rats receiving in addition to a varied high-calorie, low-protein diet, the Walser (28) ketoacid mixture, propranolol, and high doses of serine.

In the present study glycogen catabolism was prevented by killing the animals and placing them in iced water for 15 min. It can be argued that, even in the extract prepared thereafter, the measured active fractions of phosphorylase and synthetase would not resemble the *in vivo* situation. However, it must be stressed that the percentage of phosphorylase *a* activity to total phosphorylase activity was 6% in the crude extract and 8% in the protein–glycogen complex of control rats. The quick freeze approach was not possible because this technique does not deliver enough material to isolate the protein–glycogen complex.

Our data show a clear correlation between glycogen concentration in the protein–glycogen complex and the specific activity of phosphorylase phosphatase. For example, the glycogen concentration in the protein–glycogen complex of untreated control rats was 166.7 μg/mg protein with a specific activity of phosphorylase phosphatase of 10.6×10^{-3} units/mg, whereas 60 hr after binephrectomy in animals on a high-calorie diet, the Walser ketoacid mixture and propranolol, the glycogen concentration was 0.11 μg/mg protein in the complex and the specific activity of the enzyme was 0.75×10^{-3} units/mg. The finding of a decreased recovery of phosphorylase phosphatase in many of these instances may be related to the fact that the glycogen level of the tissue decreased markedly. Presumably the various enzymes of the protein–glycogen complex are present due to their binding to glycogen.

According to the studies of Hörl and Heilmeyer (30), phosphorylase phosphatase is also associated with membrane fractions of the sarcoplasmic reticulum and involved in the regulation of calcium transport ATPase. Since fragments of the sarcoplasmic reticulum are isolated in association with the protein–glycogen complex (8), the regulation of the calcium transport ATPase in the sarcoplasmic reticulum must be taken into consideration when discussing the system involved in transforming phosphorylase *a* to *b*. Moreover, phosphorylase phosphatase activity is regulated by the inhibitor-1 which can be phosphorylated or dephosphorylated (31). Since an intact protein–glycogen complex with a high specific activity of phosphorylase phosphatase can be isolated from acutely uremic animals fed with high doses of serine, a high-calorie diet, Walser ketoacid mixture, and injected with propranolol, the question arises as to what extent serine administration influences the phosphorylase phosphatase inhibitor.

In the protein–glycogen complex isolated from skeletal muscle of untreated control animals, we find 8% of phosphorylase in the active *a* form after the addition of ATP and magnesium (Fig. 1). Phosphorylase in the protein–glycogen complex isolated from acutely uremic rats fed on a high-calorie diet combined with the Walser ketoacid mixture and propranolol is maximally activated (70% of the enzyme is in the active *a* form). Furthermore phosphorylase stimulation by the addition of calcium, magnesium, and ATP is impossible (Fig. 1). Evidently, phosphorylase phosphatase is completely inactivated under these conditions (7). By feeding additional large doses of serine, however, the proportion of phosphorylase *a* is decreased to 30% and further permits the typical flash activation of phosphorylase under these circumstances (Fig. 1).

In acute uremia, it is unlikely that ele-

vated catecholamine levels are the only reason for stimulation of phosphorylase *a* in the protein–glycogen complex since the administration of a β-adrenergic blocker does not affect the transformation of phosphorylase *b* to *a* (7). The close correlation between glycogen concentration and phosphorylase phosphatase activity, however, suggests a regulatory mechanism via phosphorylase phosphatase in acute uremia. Phosphorylase *b* to *a* transformation in acute uremia could also be brought about in the following three ways: (i) A calcium-induced stimulation of phosphorylase kinase with synchronized inactivation of phosphorylase phosphatase; (ii) an inactivation of phosphorylase phosphatase through interaction with the sarcoplasmic reticulum; (iii) an activation of phosphorylase by a catecholamine-induced phosphorylation of phosphatase inhibitor-1 (32–35). Glycogenolysis caused by acute uremia is specifically different from a purely stress-induced stimulation of glycogen breakdown. Our data suggest that glycogenolysis in acute uremia may be caused at least in part by proteolytically activated phosphorylase kinase (7).

In accordance with the studies on chronic uremic patients (1) and chronic uremic rats (3) glycogen content was normal in the crude skeletal muscle extract (Table I). In contrast to animals with acute uremia glycogen content in the protein–glycogen complex of rats with chronic uremia was doubled compared to their respective untreated controls.

The favorable effects of high doses of serine on muscle glycogen metabolism that we observed during acute uremia are impressive. Synthesis of glycogen from lactate in frog and rabbit muscle has previously been demonstrated (40). Crabtree *et al.* (41) found pyruvate carboxylase, phosphoenolpyruvate carboxylase, and fructose diphosphatase activities in crude homogenates of vertebrate and invertebrate muscle. These results as well as our findings offer the possibility of gluconeogenesis from serine not only in liver and kidney but also in skeletal muscle. Treatment of animals with only serine cannot prevent glycogenolysis in acute uremia. Our data suggest, however, that the combination of high doses of serine with other amino acids may be supportive in the therapy of acute renal failure.

The authors are greatly indebted to Prof. Dr. L. M. G. Heilmeyer, Jr. (Institut of Physiologische Chemie, Ruhr-Universität Bochum) for his stimulating discussion. We acknowledge the excellent technical assistance of Mrs. M. Röder and Miss I. Henneberger.

This work was supported by the Deutsche Forschungsgemeinschaft (Ho 781/3-2).

1. Bergström J, Hultman E. Glycogen content of skeletal muscle in patients with renal failure. Acta Med Scand **186**:177–181, 1969.
2. DeFronzo RA, Jordan DT, Rowe JW, Andres R. Glucose intolerance in uremia. Quantification of pancreatic beta cells sensitivity to glucose and tissue sensitivity to insulin. J Clin Invest **62**:425–435, 1978.
3. Hörl WH, Sperling J, Heidland A. Enhanced glycogen turnover in skeletal muscle of uremic rats—Cause of uncontrolled actomyosin ATPase? Amer J Clin Nutr **31**:1861–1864, 1978.
4. Hörl WH, Heidland A. Glycogen metabolism in muscle in uremia. Amer J Clin Nutr **33**:1461–1467, 1980.
5. Matthews C, Heimberg KW, Ritz E, Agostini B, Fritzsche J, Hasselbach W. Effect of 1.25-dihydroxycholecalciferol on impaired calcium transport by the sarcoplasmic reticulum in experimental uremia. Kidney Int **11**:227–235, 1977.
6. Hörl WH, Heidland A. Enhanced proteolytic activity—Cause of protein catabolism in acute renal failure. Amer J Clin Nutr **33**:1423–1427, 1980.
7. Hörl WH, Stepinski J, Heidland A. Carbohydrate metabolism and uraemia—Mechanisms for glycogenolysis and gluconeogenesis. Klin Wochenschr **58**:1051–1064, 1980.
8. Meyer F, Heilmeyer LMG Jr, Haschke RH, Fischer EH. Control of phosphorylase activity in a muscle glycogen particle. I. Isolation and characterisation of the protein glycogen complex. J Biol Chem **245**:6642–6648, 1970.
9. Heilmeyer LMG Jr, Meyer F, Haschke RH, Fischer EH. Control of phosphorylase activity in a muscle glycogen particle. II. Activation by calcium. J Biol Chem **245**:6649–6656, 1970.
10. Haschke RH, Heilmeyer LMG Jr, Meyer F, Fischer EH. Control of phosphorylase activity in a muscle glycogen particle. III. Regulation of phosphorylase phosphatase. J Biol Chem **245**:6657–6663, 1970.
11. Helmreich E, Cori CF. Regulation of glycolysis in muscle. Advan Enzyme Regul **3**:91–107, 1965.

12. Morgan HE, Parmeggiani A. Regulation of glycogenolysis in muscle. II. Control of muscle glycogen phosphorylase activity. J Biol Chem 239:2440–2445, 1964.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurements with the folin phenol reagent. J Biol Chem 193:265–275, 1951.
14. Montgomery R. Determination of glycogen. Arch Biochem Biophys 67:378–386, 1957.
15. Hörl WH, Kittel R, Heidland A. Effects of high doses of leucine and ketoleucine on glycogen and protein metabolism in acute uremia. Amer J Clin Nutr 33:1468–1475, 1980.
16. Fiske CH, Subbarow I. The colorimetric determination of phosphorus. J Biol Chem 66:375–400, 1925.
17. Hedrick JL, Fischer EH. On the role of pyridoxal 5′-phosphate in phosphorylase. I. Absence of classical vitamin B_6-dependent enzymatic activities in muscle glycogen phosphorylase. Biochemistry 4:1337–1343, 1965.
18. Thomas JA, Schlender KK, Larner J. A rapid filter paper assay for UDP glucose-glycogen glucosyltransferase, including an improved biosynthesis of UDP-^{14}C-glucose. Anal Biochem 25:486–499, 1968.
19. Jennissen HP, Heilmeyer LMG Jr. An automated assay for phosphorylase kinase. Anal Biochem 57:118–126, 1974.
20. Cohen P. The subunit structure of rabbit skeletal muscle phosphorylase kinase and the molecular basis of its activation reactions. Eur J Biochem 34:1–14, 1973.
21. Jennissen HP, Heilmeyer LMG Jr. General aspects of hydrophobic chromatography. Adsorption and elution characteristics of some skeletal muscle enzymes. Biochemistry 14:754–759, 1975.
22. Krebs EG, Fischer EH. The isolation and crystallization of rabbit skeletal muscle phosphorylase *b*. J Biol Chem 231:65–71, 1958.
23. DeMeis L, Hasselbach W. Acetyl phosphate as substrate for Ca^{2+} uptake in skeletal muscle microsomes. J Biol Chem 246:4759–4773, 1971.
24. Hörl WH, Jennissen HP, Heilmeyer LMG Jr. Evidence for the participation of a Ca^{2+}-dependent protein kinase and a protein phosphatase in the regulation of the Ca^{2+}-transport ATPase of the sarcoplasmic reticulum. 1. Effect of inhibitors of the Ca^{2+}-dependent protein kinase and protein phosphatase. Biochemistry 17:759–766, 1978.
25. Glynn IM, Chappel B. A simple method for the preparation of ^{32}P-labelled adenosine triphosphate of high specific activity. Biochem J 90:147–149, 1964.
26. Weber K, Osborn M. The reliability of molecular weight determination by dodecyl sulfatepolyacrylamide gel electrophoresis. J Biol Chem 244:4406–4412, 1969.
27. Hickenbottom, JP. Studies on Skeletal Muscle Glycogen Synthetase. PhD thesis, University of Washington, 1967.
28. Walser M. Ketoacids in the treatment of uremia. Clin Nephrol 3:180–186, 1975.
29. Parmeggiani A, Luft JH, Love DS, Krebs EG. Cristallization and properties of rabbit skeletal muscle phosphofructo kinase. J Biol Chem 241:4625–4637, 1966.
30. Hörl WH, Heilmeyer LMG Jr. Evidence for the participation of a Ca^{2+}-dependent protein kinase and protein phosphatase in the regulation of the Ca^{2+}-transport ATPase of the sarcoplasmic reticulum. 2. Effect of phosphorylase kinase and phosphorylase phosphatase. Biochemistry 17: 766–772, 1978.
31. Foulkes JG, Cohen P. The hormonal control of glycogen metabolism. Phosphorylation of protein phosphatase inhibitor-1 in vivo in response to adrenaline. Eur J Biochem 97:251–256, 1979.
32. Heilmeyer LMG Jr, Gröschel-Stewart U, Janke U, Kilimann MW, Kohse KP, Varsanyi M. Novel aspects of skeletal muscle protein kinase and protein phosphatase regulation by Ca^{2+}. Advan Enzyme Regul 18:121–144, 1980.
33. Tao SH, Huang FL, Lynch A, Glinsmann WH. Control of rat skeletal muscle phosphorylase phosphatase activity by adrenaline. Biochem J 176:347–350, 1978.
34. Toth G, Gergely P, Parsadanian HK, Bot G. Regulation of phosphorylase phosphatase from skeletal muscle by phosphorylation of a regulator protein. Acta Biochem Biophys Acad Sci Hung 12:389–398, 1977.
35. Varsanyi M, Heilmeyer LMG Jr. Ca^{2+}-regulation of sarcoplasmic reticular protein phosphatase activity. Biochemistry 18:4869–4875, 1979.
36. Bendall JR, Taylor AA. The Meyerhof quotient and the synthesis of glycogen from lactate in frog and rabbit muscle. A reinvestigation. Biochem J 118:887–893, 1970.
37. Crabtree B, Higgins SJ, Newsholme EJ. The activities of pyruvate carboxylase, phosphoenolpyruvate carboxylase and fructose diphosphatase in muscles from vertebrates and invertebrates. Biochem J 130:391–396, 1972.

Received July 30, 1981. P.S.E.B.M. 1982, Vol. 169.