Summary. Glyceride triolein-1-14C was fed to bile fistula and sham operated rats which were killed after 4 hours. In the bile fistula animals there was a diminished absorption and decreased lipolysis of the triolein. Analysis of small gut mucosal lipid showed a greater proportion of radioactivity in the FFA and lower glycerides of the bile fistula animals than of the controls. This supports the concept that bile facilitates mucosal esterification of absorbed long chain fat.

1. Dawson, A. M., Isselbacher, K. J., J. Clin. Invest., 1960, v39, 730.

2. Saunders, D. R., Dawson, A. M., Gut. 1963, v4, 254.

3. Gallagher, N., Webb, J., Dawson, A. M., Clin. Sci., 1965, v29, 77.

4. Bruno, G. A., Christian, J. E., Analyt. Chem., 1961, v33, 650.

5. Van Zyl, J., J. Endocrinol., 1957, v16, 213.

6. Mattson, F. H., Benedict, J. H., Beck, L. W., J. Nutrition, 1954, v52, 575.

7. Annegers, J. H., Arch. intern. Med., 1965, v93, 9.

8. Cohen, B. J., Proc. Soc. Exp. Biol. and Med., 1961, v107, 40.

9. Knoebel, L. K., Ryan, J. M., Am. J. Physiol., 1963, v204, 509.

10. Raghavan, S. S., Guneta, H. S., Murphy, S. K., Ganguly, Nature, 1965, v206, 189.

11. Kern, F., Borgstrom, B., Gastroenterology, 1965, v48, 866.

12. Laok, L., Weiner, M., Fed. Proc., 1963, v22, 1334.

Received January 3, 1966. P.S.E.B.M., 1966, v122.

Alteration of Hepatic Protein Synthesis in Acute Uremia. (31062)

GERALD J. MCCORMICK, LEROY SHEAR, AND KEVIN G. BARRY
(Introduced by W. S. Gochenour, Jr.)
Department of Metabolism, Division of Medicine, Walter Reed Army Institute of Research, Washington, D. C.

Muscle wasting and protein undernutrition frequently appear as complications in patients with uremia. These disturbances may be due in part to the nutritional deficiency of the diets usually prescribed to reduce the blood urea nitrogen level (BUN). However, some evidence suggests that azotemic patients may not metabolize protein normally. Thus, in nitrogen balance studies(1,2), uremic individuals appear to require higher than normal protein intakes to maintain nitrogen balance. Impaired absorption of dietary nitrogen cannot explain the negative balance since it usually is accompanied by increased urinary nitrogen excretion. These findings could indicate either reduced utilization of nitrogen for protein synthesis or accelerated protein breakdown.

Recently developed techniques with cell-free systems(3) permit controlled study of protein synthesis *in vitro*. The present investigation using such a system was undertaken to examine the effect of uremia on protein synthesis. Results indicate that incorporation of leucine into protein by cell-free liver preparations is altered in uremic rats.

Materials and methods. Male rats of the Walter Reed strain, weighing 200 to 250 g, were used as experimental animals. They were separated into 3 experimental groups: normal fasting controls, normal non-fasting controls, and uremic fasting test animals.

The rats were maintained on Purina chow and tap water *ad libitum* preceding the experimental period. All animals in each group were anesthetized with 15 mg sodium pentobarbital given intraperitoneally. Uremia was induced in the test group by ligation of the penis. The animals were placed in individual cages and the normal fasting control and the uremic fasting test groups were given water but no food. The non-fasting controls were allowed both food and water *ad libitum*.

Approximately 48 hours after anesthesia the rats were sacrificed by decapitation. Blood was drained into beakers containing ethylenediaminetetracetate as anticoagulant and aliquots were taken and saved for subsequent

analysis. The livers were rapidly excised, blotted, and immediately placed in ice-cold medium.* The temperature thereafter was maintained at 0-4° except for incubation periods. After careful blotting, 2 g portions of liver were taken, washed with medium, minced with scissors, and homogenized in 5.0 ml of medium in a Potter-Elvehjem homogenizer with Teflon pestle (5 passes under medium pressure at 1000 rpm). The homogenate was centrifuged at 10,000 × *g* for 30 minutes, the supernatant fraction was decanted and the pellet containing cellular debris, nuclei, and mitochondria was discarded.

Amino acid incorporation studies were performed in duplicate. A 0.3 ml aliquot of homogenate supernatant was added to 0.12 ml of 0.035 M Tris-chloride buffer containing 1.0 μmole of adenosine triphosphate,† 1.0 μmole of guanosine triphosphate, and 5.0 μmoles of phosphoenolpyruvate. To this mixture was added 10 μl of pyruvate kinase suspension (100 μg), and 50 μl of L-(U-^{14}C)-leucine solution containing 1.8×10^{-3} μmole (0.4 μc). The total volume of each incubation mixture was 0.48 ml. After a 30-minute incubation at 37°, the reaction was stopped by addition of 0.5 ml of ice-cold 20% trichloroacetic acid (TCA) containing 0.1 M DL-leucine (unlabeled), followed by 4 ml of 10% TCA. Blank incubations also were done in duplicate. These contained 0.3 ml of homogenate supernatant and 0.13 ml of Tris-chloride buffer. The L-(U-^{14}C)-leucine was added after the incubation, immediately before addition of TCA. The precipitate was washed twice with 10% TCA and digested at 90° for 15 minutes in 10% TCA to hydrolyze ribonucleic acid and thus solubilize ribonucleic acid-bound leucine. It was washed once more with 10% TCA, then with single washes of ethanol, ethanol-ether (1:1), and ether. The dry residue was dissolved in 0.3 ml of 1 N NaOH with warming, transferred to a stainless steel planchet, dried, and counted in a Nuclear-Chicago gas flow beta counter with thin window Geiger-Muller tube. Variation between duplicates for the entire incorporation procedure was ± 4%. Activity contained in the blanks averaged 0.14% of the incorporated activity. Incorporation was calculated as μμmoles of L-(U-^{14}C)-leucine per gram of protein in the homogenate supernatant.

Protein determinations were done by the biuret method on ethanol-washed TCA-precipitates from triplicate 0.1 ml samples of homogenate supernatant. Control serum obtained from Hyland Laboratories, Los Angeles, Calif., was used as the standard.

Blood plasma urea nitrogen content (BUN) was measured by the micro method of Chaney and Marbach(4), with urea as the standard.

In the initial experimental design 2 uremic fasting animals were paired with a single fasting control and were anesthetized, handled and studied together. The results of 5 such experiments were tested by analysis for variance using standard statistical methods(5). Three additional fasting uremic animals and 7 non-fasting control animals were studied in separate non-paired experiments and results were analyzed by non-paired t-testing.

Free leucine levels in liver were determined in 25 additional rats which were grouped and prepared in a manner identical to that used for the incorporation studies. Seven of the 25 rats were rendered acutely uremic and fasted, 9 served as fasting normal controls and 9 others as non-fasting normal controls. Rats in each group were separated into 3 subgroups, and entire livers from the individual animals in each subgroup were removed, pooled and homogenized. Thus, 3 pooled liver samples were obtained for each experimental group. Norleucine (2.5 μmoles) was added to each homogenate pool as an internal standard, proteins were precipitated by addition of 1% picric acid, and the fil-

* Medium consisted of 0.25 M sucrose, 0.075 M KCl, 0.01 M $MgCl_2$ in 0.035 M tris(hydroxymethyl)-aminomethane, pH 7.8 (Tris-chloride buffer).

† Adenosine triphosphate (disodium salt), phosphoenolpyruvate (tricyclohexylamine salt), and pyruvate kinase (10 mg/ml suspension in ammonium sulfate) were obtained from Sigma Chemical Co., St. Louis, Mo. Guanosine triphosphate (disodium salt) was obtained from Mann Research Laboratories, New York. Uniformly labeled L-leucine (L-(U-^{14}C)-leucine) with a specific activity of 223 mc/mmole was purchased from New England Nuclear Corp., Boston, Mass.

trates were used for free leucine analysis by ion-exchange chromatography(6).

Results. The effectiveness of penile ligation in establishing acute uremia is demonstrated by the increase in BUN levels. The values of 181 to 269 mg% in the uremic group far exceeded the upper limit of the normal range for this laboratory (23.0 ± 13.8 mg%). No correlation was apparent between leucine incorporation and the level of BUN within the uremic group.

It had been anticipated that incorporation of leucine into protein might be reduced in the uremic state because of impaired hepatic protein synthesis. Surprisingly, however, the incorporation values in livers from the fasting uremic animals were higher than those in the paired fasting controls in 9 of the 10 studies ($p<.01$). Usually the incorporation was greater by a factor of two. In the 3 additional uremic rats studied without paired controls, incorporations were greater than in any of the non-uremic rats. Mean incorporation in all of the uremic rats was 6.50 $\mu\mu$moles/g of protein as compared with 3.71 $\mu\mu$moles/g of protein in the normal fasting control group ($p<.02$) (Table I).

Incorporation also was examined in unpaired experiments using normal non-fasting control animals, to allow comparison of fasting uremic and fasting control rats with those in a true normal state. The incorporation values in $\mu\mu$moles/g of protein in the non-fasting control group had a range (1.92 to 5.28) and average (3.91) which were almost identical to those in the fasting controls (2.26 to 4.85 and 3.71). The mean incorporation in the fasting uremic rats was significantly different from that in the non-fasting control group ($p<.03$).

The level of free leucine in the livers of the fasting uremic rats was 1.90 μmoles/g of protein (mean of values for the 3 pooled samples, determinations done in duplicate). Levels in the fasting and non-fasting normal groups were 1.29 and 1.86 (Table II). Mean BUN levels for the rats in these groups were 148, 11.6, and 14.4 mg%, respectively.

Discussion. The present study performed *in vitro* using cell-free liver homogenate preparations indicates that protein metabolism is altered in uremic rats. These results are consistent with previous evidence obtained *in vivo* with liver perfusion experiments(7). In those studies production of urea and the change in perfusate amino acid levels were measured in livers from uremic and control rats. The uremic livers produced more urea and took up more amino acid than did the controls. Incorporation of amino acids into protein was not determined; however, the greater uptake of amino acids by the uremic livers is consistent with the present finding of increased incorporation in the cell-free system.

The precise meaning and significance of the increased incorporation of leucine into TCA-insoluble material by uremic rat liver have not been defined in the present study. The nature of the precipitated, leucine-containing material was not identified; however, such incorporation generally is assumed to represent utilization of the labeled amino acid for protein synthesis.

The observed increase in incorporation of labeled leucine in the uremic rats might have been due to decreased endogenous levels of the amino acid (with less dilution of the labeled leucine) rather than to increased protein synthesis. This was not the case, however, since the measured tissue levels of leucine in the uremic rats were greater than those in the fasting and equal to those in the non-fasting animals. The hepatic leucine levels were less in the fasting than in the non-fasting rats, as has been reported previously(8). Therefore, the similarity of incorporation values in these two groups is further evidence that the increased incorporation seen in the uremic animals was not an artifact resulting from less dilution of label by endogenous leucine.

Results of the present study therefore suggest that the rate of hepatic protein synthesis is increased in uremia. If this disturbance is related to the negative nitrogen balance seen in patients with uremia, the overall rate of protein degradation is increased, or uremic liver may make incomplete or abnormal protein. The muscle wasting frequently seen in uremic patients suggests the further possibility that protein metabolism is not altered

TABLE I. Incorporation of L-(U-^{14}C)-Leucine ($\mu\mu$mole/g protein) into Rat Liver Protein.

Non-fasting control*	Fasting control†	Uremic†
1.92	4.46	4.41
3.53		6.29
4.66	2.26	4.00
4.44		2.87
5.28	4.45	6.15
3.25		6.21
4.27	2.51	5.25
		6.13
	4.85	8.38
		8.40
		6.30*
		8.53*
		11.56*
Mean 3.91	3.71	6.50
S.E.M. .45	.49	1.30

* Unpaired experiments.
† Paired experiments—one control was studied simultaneously with 2 uremic animals.

TABLE II. Endogenous Leucine in Liver, μmoles/g of Protein.

Sample*	Uremic	Fasting normal	Non-fasting normal
1	2.43	1.32	2.05
2	1.29†	1.02	1.88
3	1.71†	1.52	1.64
Mean	1.90‡	1.29	1.86

* Livers from 3 animals were pooled to make each sample.
† Livers from 2 rather than 3 animals.
‡ Weighted mean.

in the same manner in all body tissues. Thus, the liver could synthesize protein at an increased rate in uremia, while muscle and other tissues exhibit increased rates of degradation.

Results obtained in cell-free systems cannot be applied directly to the intact animal. Nevertheless, utilization of such techniques permits investigation of protein metabolism with far better control than is possible in uremic patients. Further studies are in progress to better define the abnormality in protein synthesis observed in the present study and to investigate its relationship to amino acid metabolism.

Summary. Incorporation of L-(U-^{14}C)-leucine into protein was examined *in vitro* using cell-free preparations of liver from fasting acutely uremic, fasting normal, and non-fasting normal rats. The amount of incorporation was similar in the fasting and non-fasting normals. Incorporation by the acutely uremic liver preparations was significantly greater than by either control group. The measured hepatic leucine levels indicated that these findings cannot be ascribed to less dilution of label by endogenous free leucine. These results indicate that hepatic protein synthesis in rats is altered in uremia.

The authors gratefully acknowledge the assistance of Mr. Lloyd Clayton in preparation of the animals, Mr. Ethelbert Dawson in determination of leucine levels, and Mrs. Doris Blackmon and Mrs. Marian Kulak who provided secretarial assistance.

1. Olbrich, O., Edinburgh Med. J., 1944, v51, 327.
2. Herndon, R. F., Freeman, S., Cleveland, A. S., J. Lab. Clin. Med., 1958, v52, 235.
3. Pena, A., Dvorkin, B., White, A., Biochem. and Biophys. Res. Commun., 1964, v16, 449.
4. Chaney, A. L., Marbach, E. P., Clin. Chem., 1962, v8, 130.
5. Goulden, C. H., Methods of Statistical Analysis, 2nd Ed., John Wiley & Sons, Inc., New York, 1952, pp50-101.
6. Spackman, D. H., Stein, W. H., Moore, S., Anal. Chem., 1958, v30, 1190.
7. Lacy, W. W., Clin. Res., 1965, v13, 80.
8. Allison, J. B., Wannemacher, R. W., Jr., Banks, W. L., Jr., Fed. Proc., 1963, v22, 1126.

Received January 3, 1966. P.S.E.B.M., 1966, v122.