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Research Article

When normal individuals eat 0.33 g protein N/kg body weight (BW)$^{3/4}$ per day, they excrete 10-15 mg urea N/h per kg BW$^{3/4}$. If they now ingest (at 0 h) 0.27 (dose A), 0.40 (dose B), 0.53 (dose C), 0.94 (dose D), or 1.33 (dose E) g protein N/kg BW$^{3/4}$ (in the form of casein, ovalbumin, or lactalbumin), the rate of urea N excretion accelerates within 4 h. At dose C a maximal rate of urinary urea N excretion (MRUE) is reached, which averages 55 mg urea N/h per kg BW$^{3/4}$ and which persists for 16 h. Higher doses of protein do not further accelerate urea excretion, but prolong the duration of MRUE to 28 h (after dose E). Blood urea N (BUN) rises by 7-20 mg/100 ml during the first 8 h after dose C to E, and remains stable within ±5 mg/100 ml during the ensuing 8-28 h of MRUE. Each increment of protein above dose C causes a further increment in plasma α-amino N. During infusion of free amino acids at a rate of 110 or 165 mg amino acid N/h per kg BW$^{3/4}$ for 12 h, rate of urea excretion increases to the MRUE value produced by dose C-E of oral protein.

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Maximal Rates of Excretion and Synthesis of Urea in Normal and Cirrhotic Subjects

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ABSTRACT When normal individuals eat 0.33 g protein N/kg body weight (BW)$^{3/4}$ per day, they excrete 10–15 mg urea N/h per kg BW$^{3/4}$. If they now ingest (at 0 h) 0.27 (dose A), 0.40 (dose B), 0.53 (dose C), 0.94 (dose D), or 1.33 (dose E) g protein N/kg BW$^{3/4}$ (in the form of casein, ovalbumin, or lactalbumin), the rate of urea N excretion accelerates within 4 h. At dose C a maximal rate of urinary urea N excretion (MRUE) is reached, which averages 55 mg urea N/h per kg BW$^{3/4}$ and which persists for 16 h. Higher doses of protein do not further accelerate urea excretion, but prolong the duration of MRUE to 28 h (after dose E). Blood urea N (BUN) rises by 7–20 mg/100 ml during the first 8 h after dose C to E, and remains stable within ±5 mg/100 ml during the ensuing 8–28 h of MRUE. Each increment of protein above dose C causes a further increment in plasma α-amino N. During infusion of free amino acids at a rate of 110 or 165 mg amino acid N/h per kg BW$^{3/4}$ for 12 h, rate of urea excretion increases to the MRUE value produced by dose C-E of oral protein.

These findings indicate that MRUE corresponds to a period of maximal rate of urea synthesis (MRUS). MRUS is greater than MRUE because one fraction of newly formed urea is hydrolyzed in the gastrointestinal tract, and another fraction may accumulate temporarily in body water during the MRUE period. Oral neomycin reduces the proportion of urea hydrolyzed in the gut to less than 20%; its extent is measured by recovery in the urine of a tracer dose of [^{14}C]urea injected intramuscularly during determination of MRUE. Accumulation of urea in body water is estimated from increment in BUN during the period of MRUE measurement (8–24 h after dose E of casein) and from body water measured with $^{3}H_2O$. Then MRUS is calculated as: ([mg urea N excreted between 8 and 24 h after dose E] + [BUN at 24 h − BUN at 8 h] × [body water]) × (100/% recovery [^{14}C]urea) × (1/kg BW$^{3/4}$) × (1/16 h).

MRUS in 10 normal subjects averaged 65 mg urea N/h per kg BW$^{3/4}$ (range 55–76), and in 34 cirrhotics 27 mg urea N/h per kg BW$^{3/4}$ (range 6–64). Among 19 cirrhotic patients fed 40, 60, 80, or 100 g protein daily for successive 10 day periods, the occurrences of hyperammonemia, hyperaminoacidemia, and encephalopathy at each level of protein intake were inversely related to MRUS value.

INTRODUCTION

Hepatic enzymes incorporate NH_3 and the amino groups of amino acids into urea, the principal end-product of N metabolism. In normal subjects, the rate of urea production is known to be proportional to protein intake, but the maximal rate which can be achieved has not been measured. In cirrhotic patients, a reduced capacity to synthesize urea is suggested by elevated concentrations of NH_3 and amino acids, and by depressed concentration of urea, in blood or plasma. But in these patients, too, the maximal rate has not been measured, so the extent of this impairment is not known.

Preliminary experiments have shown that a maximal rate of urea N excretion (MRUE) [1] in a given individual can readily be achieved and maintained for 16 h or longer by administering a suitable load of oral protein or of intravenous amino acids. Before one can

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[1] *Abbreviations used in this paper:* AAN, plasma α-amino nitrogen; BUN, blood urea nitrogen; BW, body weight; MRUE, maximal rate of urinary urea N excretion; MRUS, maximal rate of urea synthesis.

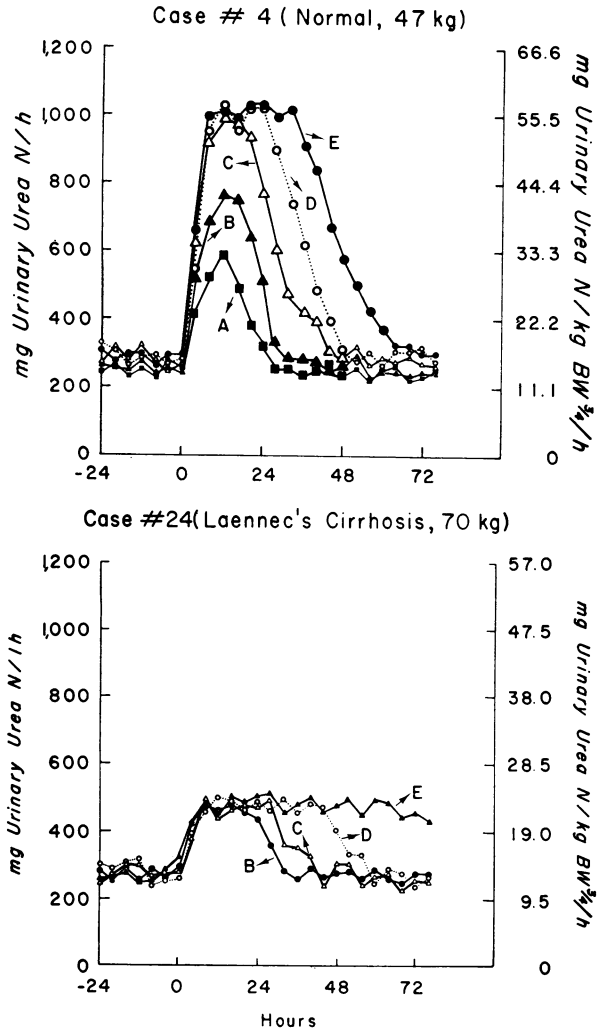

FIGURE 1 Rate of urinary excretion of urea N in two subjects. Beginning at −72 h, and continuing from 0 to 96 h, patient was fed the "basic" diet. At 24 h, dose A, B, C, D, or E of casein was administered. (For doses D or E, a portion of the dose was ingested at 28, or at 28 and 32 h, respectively). Experiments involving each dose were done at 4–8 day intervals.

calculate the maximal rate of urea N synthesis (MRUS) corresponding to MRUE, two complications must be considered: (*1*) The major proportion of urea synthesized during a 16 h interval is excreted in the urine, but a minor proportion diffuses through the body H_2O and its excretion may be delayed. (*2*) An additional proportion is hydrolyzed within the gastrointestinal tract by microbial ureases. McKinley, Gilbert, Chao, and Reeve (1) and Walser and Bodenlos (2) have developed methods to estimate both factors: (*a*) The urea space can be measured with [^{14}C]urea or ^3H_2O, and the increment of urea in body water during the period of observation calculated as product of increment in blood urea N concentration (BUN) and urea space; (*b*) hydrolysis of urea in the gut can be minimized by antibiotics, and the extent of residual hydrolysis estimated from the urinary recovery of a tracer dose of parenterally administered [^{14}C]urea.

The objectives of the present study were: (*a*) to establish conditions under which MRUE occurs; (*b*) to determine MRUE in normal and cirrhotic subjects; (*c*) by the method of McKinley et al. (1), to calculate MRUS from MRUE, BUN, body water, and recovery of [^{14}C]urea; (*d*) to correlate reduction in MRUS in cirrhotic patients with other manifestations of their liver disease.

METHODS

Subjects were 34 patients with biopsy-proven cirrhosis and 10 normal individuals. In 21 cirrhotic patients, shunt surgery had been performed. Nine of these 21 were studied before, and all after the operation. Etiologic diagnoses were: alcoholic cirrhosis, 29; postnecrotic cirrhosis, 2; biliary cirrhosis, Wilson's disease, and cirrhosis secondary to ulcerative colitis, 1 each. An appendix deposited with National Auxiliary Publication Service gives a clinical summary of each subject. Tests of urea synthesis developed in this investigation are influenced by size of body water space and by renal function. In the present study, patients with ascites, edema, or impaired renal function were excluded. In all 44 subjects, fasting BUN was less than 20 mg/100 ml, plasma creatinine concentration was less than 1.0 mg/100 ml, and creatinine clearance was greater than 80 ml/min per 1.73 m^2 surface area.

Patients received the following daily diet divided into four equal aliquots at 6-h intervals beginning at 8 a.m.: 2 g protein (containing 0.33 g N)/kg body weight (BW)$^{3/4}$; 10 g carbohydrate/kg BW$^{3/4}$; 4 g fat/kg BW$^{3/4}$ (henceforth termed "basic diet"). Each feeding included 200 ml of protein-free formula diet (3). At 4–8 day intervals, an "oral" or "intravenous" experiment was done. In oral experiments, a purified protein (casein, lactalbumin, or ovalbumin [ICN Nutritional Biochemicals Div., Cleveland, Ohio]) was introduced into the formula at 8 a.m. and sometimes at noon and 4 p.m. In intravenous experiments, a solution containing 8.5 g/100 ml of a mixture of 14 L-amino acids (Freamine, McGaw Laboratories Inc., Glendale, Calif.) was infused for 12 h, beginning at 8 a.m., at a rate of either 110 or 165 mg amino acid N/h per kg BW$^{3/4}$.

Experiments were conducted with informed written consent of the subjects. To insure complete intake of prescribed nutrition, they ate under observation.

Urine was collected in 4-h pools and analyzed as described under Results. Body water was measured with ^3H_2O by the method of Prentice et al. (4). The following references describe analytic methods: urine urea N (5); BUN (6); blood NH_3 (7); plasma α-amino nitrogen (AAN) (8). On specified days, 4 μCi (0.1 mg) [^{14}C]urea or 4 μCi NaH-$^{14}CO_3$ (0.2 mg) (New England Nuclear Corp., Boston, Mass.), dissolved in 2 ml 0.9% NaCl and sterilized by Nalge filter, was injected intramuscularly and ^{14}C content of urinary urea was determined for 72 h according to Walser and Bodenlos (2).

TABLE I

Magnitude and Duration of Peak Excretory Rate of Urea in Six Normal Subjects and in Six Cirrhotic Patients after Doses A–E of Casein, Lactalbumin, or Ovalbumin

Protein	Dose	Peak excretory rate (8–24 h)		Duration of peak excretory rate		Plasma α-amino N at 24 h	
		Normal	Cirrhosis	Normal	Cirrhosis	Normal	Cirrhosis
		mg urea N/h/kg BW$^{3/4}$		*h*		*mg/100 ml*	
Casein	A	32.6±3.1	13.4±2.1*	4	12	8.1±0.8	9.1±0.8
	B	38.2±2.2	16.5±3.0*	8	16	9.6±0.9	11.5±0.7
	C	53.1±4.3	22.4±2.7*	8	24	9.0±1.1	12.4±1.6
	D	50.2±5.7	23.0±4.1‡	20	40	11.6±1.6	13.7±1.3
	E	53.5±3.4	22.6±3.6*	28	88	12.4±1.2	14.5±2.1
Lactalbumin	C	43.2±5.2	22.6±2.4‡	8	20	10.1±0.8	11.7±1.4
	D	54.6±3.9	19.4±3.1*	16	36	11.6±1.5	13.6±1.0
	E	52.0±3.5	23.7±1.3*	28	78	13.0±1.3	14.3±1.3
Ovalbumin	C	55.4±3.2	21.1±2.6*	8	20	9.4±0.8	9.9±1.4
	D	51.6±3.3	23.4±3.2*	16	30	11.6±1.4	13.0±1.1
	E	53.0±5.5	24.1±3.9‡	34	82	13.4±0.7	14.3±2.0

‡ Values for excretory rate and α-amino N are average ±SE. Each of these values for cirrhotic group was compared with corresponding value of normal group. *P* values less than 0.05 are indicated as follows.
* <0.001.
‡ <0.005.

Preliminary experiments

Normal subjects. These individuals ate the "basic diet" and received 2 g neomycin orally every 8 h. By day 3, their rate of urea excretion had stabilized at 10–15 mg urea N/h per kg BW$^{3/4}$. On day 4, a protein supplement was added to the formula according to this schedule: dose A, 0.27 g protein N/kg BW$^{3/4}$ at 8 a.m.; dose B, 0.40 g protein N/kg BW$^{3/4}$ at 8 a.m.; dose C, 0.53 g protein N/kg BW$^{3/4}$ at 8 a.m.; dose D, 0.53 g protein N/kg BW$^{3/4}$ at 8 a.m. and 0.40 g protein N/kg BW$^{3/4}$ at noon; dose E, 0.53 g protein N/kg BW$^{3/4}$ at 8 a.m., 0.40 g protein N/kg BW$^{3/4}$ at noon, and 0.40 g protein N/kg BW$^{3/4}$ at 4 p.m.[2] After each dose, the rate of urea excretion accelerated (Fig. 1). Progression from Dose A–C increased peak rate of excretion; after doses D and E, peak rate was not further increased but its duration was progressively lengthened. Peak rate after dose E, which persisted for 20–28 h, was termed the maximal rate of urea excretion (MRUE). Casein, lactalbumin, and ovalbumin influenced urea excretion in the same way (Table I). After dose E of each protein, average MRUE of six normal individuals was 50–55 mg urea N/h per kg BW$^{3/4}$; for each protein, this rate was maintained 20 h or longer.

During the period of MRUE after dose E, BUN was generally stable (±5 mg/100 ml) at a level 5–12 mg/100 ml higher than before the protein supplement (Table II). Blood NH_3 concentration did not rise. Plasma AAN (normal fasting range, 6.0–7.5 mg/100 ml) rose progressively, reaching a peak of 10.1–13.4 mg/100 ml at 24 h (Table I).

[2] For a 70 kg subject these doses are: A, 40 g protein; B, 60 g protein; C, 80 g protein; D, 140 g protein; E, 200 g protein. In experiments involving dose E in four normal and in six cirrhotic subjects weighing 51–62 kg, stools collected during the 72 h (*a*) before and (*b*) after 8 a.m., day 4, were analyzed for N. In each case, N content of collection (*b*) minus that of collection (*a*) was less than 2 g. Thus, more than 92% of dose E had been absorbed.

TABLE II

Measurement of MRUE and Calculation of MRUS in Case No. 23

Time	Urinary urea N	BUN at beginning of each 4 h interval	Urinary [^{14}C] urea
	mg/h	*mg/100 ml*	*% recovered*
0–4 h	219	12	28
4–8 h	392	15	20
8–12 h	444	16	17
12–16 h	416	15	10
16–20 h	463	17	7
20–24 h	447	17	5
24–36 h	418	18	3
36–48 h	450	14	0
48–60 h	390	15	0
60–72 h	367	12	0
72–84 h	263	14	0
84–96 h	195	9	0

BW = 51 kg; BW$^{3/4}$ = 19.0 kg; body water = 33.0 liters; urea N excretion (hours 8–24) = 7,070 mg; MRUE = 23.3 mg urea N/h per kg BW$^{3/4}$; increment of urea N in body water (hours 8–24) = (18–16) (330) = 660 mg; [^{14}C]urea recovery = 90%; MRUS = (7,070 + 660) (100/90) × (1/16) × (1/19.0) = 28.3 mg urea N/h per kg BW$^{3/4}$.

TABLE III

Comparison between Rates of Urinary Urea Excretion, Blood NH_3, AAN,

Hours of exp.	Casein								Freamine			
									110 mg N/h/kg $BW^{3/4}$			
	Excretory rate		NH_3		AAN		BUN		Excretory rate		NH_3	
	Nor	Cir	Nor	Cir	Nor	Cir	Nor	Cir	Nor	Cir	Nor	Cir
	mg urea N/h/kg $BW^{3/4}$		*μg/100 ml*		*mg/100 ml*		*mg/100 ml*		*mg urea N/h/kg $BW^{3/4}$*		*μg/100 ml*	
0–4	12.3±0.9	12.6±1.7	48±8	65±10	6.3±0.8	6.6±0.7	12±0.6	10.1±1.1	11.3±2.4	12.0±1.8	40±7	70±6*
4–8	30.4±2.6	18.7±1.4§	52±5	150±12‖	8.5±0.9	8.1±0.7	18±0.5	14.5±1.1‡	35.4±3.0	17.7±2.7§	45±8	140±19‖
8–12	51.6±4.3	23.0±3.1‖	40±7	185±26‖	9.0±1.1	10.1±1.3	20±0.9	15.4±1.2‡	55.4±4.6	20.4±3.3‖	42±6	190±20‖
12–16	54.5±2.8	21.6±1.7‖	60±6	160±18‖	11.1±0.8	12.4±0.9	19±0.8	13.7±0.9§	52.3±5.2	23.9±2.0‖	45±4	160±20‖
16–20	52.7±2.8	24.5±2.4‖	40±8	175±21‖	11.5±1.6	15.1±1.3	22±1.0	15.1±1.1‖	53.5±6.7	22.6±1.1§	39±5	180±28‖
20–24	50.6±3.9	22.0±3.0‖	45±5	160±23‖	12.4±1.2	13.6±1.8	18±0.6	15.1±1.2	35.3±2.0	22.4±2.6§	50±8	180±22‖

Values are average ±SE (n = 6 for normals [nor] and 5 for cirrhotics [cir]). Each value of the cirrhotic group was compared with the corresponding value of the normal group. *P* values less than 0.05 are indicated as follows:

* <0.01.

‡ <0.05.

§ <0.005.

‖ <0.001.

Each dose of protein (A–E) caused a progressively higher peak in plasma AAN (Table I).

The period of MRUE, accompanied by a stable BUN, could correspond to a maximal rate of urea synthesis in the liver, or to a maximal rate of protein digestion-amino acid absorption in the gastrointestinal tract. To clarify this point, two intravenous experiments were done in each of six normal subjects (Table III). The amino acid mixture was infused for 12 h at a rate either two times (first experiment) or three times (second experiment) the average normal MRUE, i.e., at a rate of 110 or 165 mg amino acid N/h per kg $BW^{3/4}$. A maximal rate of urea excretion was established by 8 h and persisted for 12–16 h; in each individual, this rate in both first and second intravenous experiment was within ±15% of the MRUE value observed after oral dose E of casein. In six normal subjects, MRUE determined (*a*) by dose E of casein, (*b*) by infusion of 110 mg amino acid N/h per kg $BW^{3/4}$, (*c*) by infusion of 165 mg amino acid N/h per kg $BW^{3/4}$, did not differ significantly ($P < 0.05$) [3] (Table III).

Cirrhotic patients. In six cirrhotic patients selected for preliminary study (Table I), relationships between oral protein supplement, urea excretion, AAN, and NH_3 differed from those in healthy individuals in these respects: (*a*) MRUE was achieved at a lower dose of protein (usually dose B or C) than in normal subjects (Fig. 1, Table I). (*b*) MRUE averaged 40% of normal ($P < 0.005$). (*c*) MRUE at a specified dose of protein (B–E) lasted two to four times longer than in normal individuals (Table I). (*d*) Peak elevation in plasma AAN at a specified dose of protein was 10–30% greater than in the normal group (Table I), but the difference was not significant at 0.05 level. (*e*) Blood NH_3 concentration, which did not increase significantly after dose E in the normal individuals, rose by 80–250 μg/100 ml in the cirrhotic subjects (Table III) ($P < 0.001$ for difference between normals and cirrhotics).

We concluded that in both normal and cirrhotic subjects, the period of MRUE after oral ingestion of protein at dose E corresponded to a period of MRUS. But the observed MRUE must have been lower than the simultaneous MRUS because of (*1*) the temporary accumulation, in some experiments, of a small [4] proportion of newly synthesized urea in body water, and (*2*) destruction of a small [5] proportion of newly synthesized urea by intestinal bacteria. These factors can be estimated, as shown by McKinley et al. (1), by measuring (*a*) body H_2O space; (*b*) change in BUN during the experiment; and (*c*) recovery in the urine of a tracer dose of [^{14}C]urea injected during the period of MRUE. Change in quantity of urea N in body water [6] during a given period of observation equals (BUN [mg/100 ml] at end of period − BUN at beginning) × (body water [in units of 100 ml]). Percent recovery of [^{14}C]urea equals (μCi ^{14}C recovered in urine urea × 100)/(μCi ^{14}C urea injected). The interval 8–24 h after beginning ingestion of dose E casein was selected as the period for measuring MRUE (Fig. 1). Now, MRUS = ([mg

[3] Unless otherwise stated, *P* values were calculated by Student's *t* test (both tails).

[4] The change in quantity of urea in body H_2O between 8 and 24 h in normal and cirrhotic subjects, calculated as product of change in BUN and body water space, averaged +7% and +5% respectively, of the amount excreted in the urine during that interval.

[5] The data of Walser and Bodenlos (2) indicate that under neomycin treatment, 80–100% of newly synthesized urea escapes hydrolysis in the gastrointestinal tract and is excreted directly in the urine.

[6] The experiments of Painter (9), Foy and Schneider (10), Bradbury (11), and Williams et al. (12), show that the urea space, the body water space measured by tritiated or deuterated water, and the body water space measured by desiccation, agree within ±5%. In the present study with subjects whose renal function was normal, the amount of newly synthesized urea which accumulated in body water during the period (8–24 h) when MRUE was measured was less than 15% of the amount of urea excreted in the urine during the same period. Therefore a possible discrepancy of 5% between urea space and tritiated H_2O space in these subjects would cause an error of less than 1% in calculated MRUS value. In patients with impaired renal function, a discrepancy between urea space and tritiated H_2O space may lead to a larger error in calculated MRUS.

and BUN after Dose E of Casein and during Infusion of Freamine

Freamine											
110 mg N/h/kg $BW^{3/4}$				165 mg N/h/kg $BW^{3/4}$							
AAN		BUN		Excretory rate		NH_3		AAN		BUN	
Nor	Cir	Nor	Cir	Nor	Cir	Nor	Cir	Nor	Cir	Nor	Cir
mg/100 ml		*mg/100 ml*		*mg urea N/h/kg $BW^{3/4}$*		*μg/100 ml*		*mg/100 ml*		*mg/100 ml*	
6.0±0.7	7.1±0.8	11±1.1	10±0.8	13.0±2.2	11.7±1.0	40±5	70±9‡	6.6±0.8	7.1±0.9	11±0.8	11±0.7
8.7±0.9	9.0±0.9	16±0.8	12±0.6§	40.3±3.5	18.1±1.5‖	50±8	150±20‖	8.5±0.8	10.2±0.9	18±1.3	15±0.8
10.8±1.3	12.5±1.7	20±1.3	18±0.7	57.2±4.9	24.0±2.3‖	40±11	220±25‖	10.4±0.9	12.7±1.4	23±1.1	20±1.0
13.0±1.0	13.1±1.4	22±1.3	16±0.5‡	56.8±4.6	22.2±2.8‖	55±9	210±26‖	14.0±1.1	15.3±1.7	21±1.4	19±1.2
12.2±1.6	14.4±1.5	21±1.8	18±1.3	55.0±4.4	20.9±3.5‖	60±12	230±30‖	13.8±1.6	15.9±1.7	24±2.0	21±1.3
9.1±1.8	13.0±1.1	17±1.0	14±0.9	48.1±3.4	20.5±2.5‖	50±5	210±18‖	11.2±1.6	14.6±1.5	18±1.9	20±1.1

urea N excreted between 8 and 24 h] + [$BUN_{24\,h} - BUN_{8\,h}$] [body water]) × (100/% recovery [^{14}C]urea) × (1/kg $BW^{3/4}$) × (1/16 h).

As noted by Walser and Bodenlos (2), this equation assumes that $^{14}CO_2$ produced by hydrolysis of [^{14}C]urea is not reincorporated into urea. The assumption was verified by injecting 4 μCi $NaH^{14}CO_3$ intramuscularly in three control and in three cirrhotic subjects and monitoring urinary urea for ^{14}C content for 48 h. No radioactivity was detected in urinary urea.

Protocol for measuring MRUS

For 9 days, subjects ate the "basic diet" and received 2.0 g neomycin at 8-h intervals. Urea N was measured in each 4 h urine collection. On day 4, BUN was determined at 8 a.m. and dose E of casein was ingested; 4 μCi [^{14}C]urea was injected intramuscularly. BUN was measured at 6-h intervals for the next 24 h. Urine urea ^{14}C counts were measured in each 4 h urine collection through day 5. On day 8, body H_2O space was determined. The data and calculations of a typical experiment are shown in Table II.

MRUS in normal and cirrhotic subjects

MRUS averaged 65 mg urea N/h per kg $BW^{3/4}$ (range 55–76) in 10 normal subjects, and 27 mg urea N/h per kg $BW^{3/4}$ (range 6–64) in 34 cirrhotic subjects (Table IV); P was < 0.001 for the difference between the two groups. MRUS was significantly ($P < 0.001$) lower in 10 cirrhotics with a history of encephalopathy (average ±SE, 15±1.5 mg urea N/h per kg $BW^{3/4}$) than in 24 without such a history (average ±SE, 31±2.2 mg urea N/h per kg $BW^{3/4}$). In nine cirrhotic patients studied before and after shunt surgery, MRUS was reduced 0–44% by the operation (Table V). (MRUS values of individual patients are given in the appendix deposited with National Auxiliary Publication Service.)[7]

[7] ASIS/NAPS document no. 02127, c/o Microfiche Publications, New York.

Other observations on NH_3 and amino acid metabolism in the cirrhotic subjects

Since MRUS in the cirrhotics ranged from 10 to 90% of normal, it was of interest to correlate this value with other clinical tests of NH_3 and amino acid metabolism. (*a*) After 4 days on a 40 g protein diet, neomycin being withheld, the patient was fasted overnight. At 8 a.m. blood NH_3 concentration was measured and a standard dose of NH_4Cl was ingested. Blood NH_3 was determined again at 10 a.m. The doses of NH_4Cl were: 0.0025, 0.0050, 0.010, 0.021, 0.042, 0.084, 0.13, 0.17, and 0.21 g/kg $BW^{3/4}$. (For a 70 kg subject, these doses are 0.063, 0.125, 0.25, 0.5, 1, 2, 3, 4, and 5 g, respectively.) The smallest dose of NH_4Cl which caused an increment of 40 μg/100 ml or higher in blood NH_3 concentration was termed the minimal effective dose (MED) required to produce hyperammonemia. (*b*) 19 cirrhotic subjects were observed on a 40 g protein diet for 10 days without neomycin. On days 8–10, electroencephalogram (EEG) and neurologic status were graded according to Parsons-Smith, Summerskill, Dawson, and Sherlock (13); these were grade 0, A, or B for EEG and grade 0 or I for neurologic status. AAN and NH_3 were also measured. The patients were then observed for three successive periods of 10 days (or less, if hepatic encephalopathy was precipitated) on a diet of 60, 80, and of 100 g protein daily, neomycin being withheld. On days 7–10, or sooner if encephalopathy occurred (as manifested by EEG of grade C, D, or E and neurologic status of grade III or IV), the EEG, fasting blood NH_3 and plasma AAN were measured.

The results are given in Tables IV and VI, where the patients are arranged in groups I, II, III, IV, and V according to MRUS value > 50, 40–50, 30–40, 20–30, or < 20 mg urea N/h per kg $BW^{3/4}$, respectively. Individuals of groups I and II tolerated 100 g protein daily with no sign of encephalopathy and with little or no rise in fasting blood NH_3 or AAN. In these groups, MED of NH_4Cl was > 0.21 g/kg $BW^{3/4}$. Individuals of group V developed hepatic encephalopathy within 7–10 days on 60 g protein intake, with simultaneous hyperammonemia and hyperaminoacidemia. Their fasting blood NH_3 concentration was fre-

TABLE IV

Relations between Clinical Status, MRUS Value,

Category	Group	Number of patients*	Age range	Percent of cases with history of encephalopathy	Percent of cases with shunt	Albumin
						g/100 ml
Normal		10	18–54	0	0	4.2±0.8
Cirrhosis	I–V	42	20–64	23	49	3.5±0.2
Cirrhosis	I	4	39–54	0	20	3.6±0.2
Cirrhosis	II	5	55–64	0	60	4.0±0.1
Cirrhosis	III	3	41–64	0	100	3.2±0.4
Cirrhosis	IV	15	33–59	0	33	3.2±0.1
Cirrhosis	V	15	20–60	67	60	3.4±0.1

Within the cirrhotic category, patients were classified into groups I–V on the basis of MRUS: Group I, >50 mg urea N/h per kg $BW^{3/4}$; Group II, 40–50; Group III, 30–40; Group IV, 20–30; Group V, <20. Values in last seven columns represent average ±SE. Correlation coefficients (r) and their P values were as follows: MRUS vs. sensitivity to NH_4Cl, $r = 0.89$, $P = 0.001$; MRUS vs. bilirubin concentration, $r = 0.06$, $P = 0.07$; MRUS vs. hemoglobin concentration, $r = 0.02$, $P = 0.9$; MRUS vs. albumin concentration, $r = 0.27$, $P = 0.1$; MRUS vs. prothrombin time, $r = -0.23$, $P = 0.2$.

* Cirrhotic patients studied before and after shunt surgery are considered twice.

‡ Value varies between 0.005 and >0.21.

§ Average of two observations.

quently elevated on the 40 g protein diet. The amount of NH_4Cl required to increase blood NH_3 by 40 μg/100 ml averaged 0.033 g/kg $BW^{3/4}$. Cirrhotic subjects in groups III and IV were intermediate between groups I and V in these respects.

Within the cirrhotic category, the correlation between MRUS and sensitivity to NH_4Cl was significant at the 0.01 level (Table IV). MRUS showed little or no correlation with concentrations of albumin, bilirubin, or hemoglobin (Table IV).

DISCUSSION

The MRUE which occurs after oral ingestion of protein at doses C → E in normals and at doses B → E in cirrhotics (Fig. 1) could be determined by (*1*) the maximal capacity to synthesize urea in the liver, or (*2*) the maximal capacity to digest protein and absorb amino acids in the gastrointestinal tract. Proof of (*1*) is: (*a*) a progressively greater accumulation of plasma amino acids with each increment in oral protein through the entire range of doses, including those greater than the dose required to establish MRUE; (*b*) equivalence of MRUE value during infusion of amino acids to that produced by dose E of casein (Table IV). If the MRUE value after dose E were determined by rate of protein digestion-amino acid absorption in the gut, then the urea excretory values during infusion of amino acids, at the infusion rates used, would have exceeded the values after dose E orally. Furthermore, increments in oral dose of protein after MRUE had been attained would not have led to further increments in plasma AAN, as in the experiments of Table I.

In the cirrhotic subjects, MRUS ranged from 10 to 90% of normal. A healthy person of 50 kg BW can convert each day into urea the N of 190 g protein, but group V cirrhotic patients can convert the N of only 20–60 g protein. Where is the block in urea synthesis? The steps by which the N of plasma amino acids is converted to urea N are shown in Fig. 2. In normal subjects, during the period of MRUS, amino acids accumulate in plasma but NH_3 does not. Therefore, the rate-limiting steps in urea synthesis in normal individuals must be transport of amino acids from extra- to intracellular space, or enzymatic processes I, II, or III of Fig. 2. In patients with chronic liver disease, the even greater accumulation of plasma amino acids during the period of MRUS (Table IV) suggests impaired transport of amino acids into liver cells (which could result from reduction of portal blood flow, or from dysfunction of cellular transport mechanisms, or both), or reduced activity of the hepatic enzymes responsible for processes I, II, and III in Fig. 2. Simultaneous accumulation of NH_3 in these patients indicates additional rate-limiting step(s) which had not been saturated by dose E in the normals: either VI, VII, IX, or XI (Fig. 2). The activities of the enzymes which catalyze I, II, III, V, and VII will need to be compared in normal and cirrhotic livers in order to distinguish these possibilities.

Sensitivity to NH_4Cl, and History of Encephalopathy

Bilirubin	Prothrombin time	Hemoglobin	Sensitivity to NH_4Cl	MRUS	Creatinine clearance
mg/100 ml	*s*	*g/100 ml*	MED in *g/kg* $BW^{3/4}$	*mg urea N/h/kg* $BW^{3/4}$	*ml/min/1.73 m² surface area*
0.4±0.04	11.1±0.3	14.3±0.2	>7.21	65.0±2.9	99±7
2.8±0.4	13.3±0.5	12.6±0.9	‡	27.0±2.5	103±2
2.3±0.5	12.6±0.9	11.6±0.7	>0.21	55.9±2.3	101±7
1.4±0.2	11.9±0.4	12.8±0.9	0.20±0.015	39.7±2.1	94±7
5.4±0.8	15.2§	14.1±2.1	0.10±0.015	35.0±2.2	112±9
2.7±0.3	13.3±0.6	12.5±0.5	0.078±0.023	25.2±0.8	103±4
2.3±0.3	13.4±0.5	12.2±0.5	0.033±0.008	15.5±0.9	101±4

TABLE V

*Effect of Shunt Surgery on MRUS and Sensitivity to NH_4Cl**

Case no.	Type of shunt	MRUS			NH_4Cl Sensitivity	
		Before shunt	After shunt	Change	Before shunt	After shunt
		mg urea N/h/kg $BW^{3/4}$		%	*g/kg* $BW^{3/4}$	
12	Side to side Mesorenal	56.1	31.4	−44	>0.21	0.010
14	Distal Splenorenal	56.3	51.8	−8	>0.21	>0.21
19	Distal Splenorenal	33.1	34.5	+4	0.13	0.084
15	Distal Splenorenal	45.4	42.7	−6	0.084	0.084
20	Dacron "H" Mesocaval	29.8	17.0	−43	0.13	0.021
23	Side to side Mesorenal	30.1	26.4	−12	0.021	0.042
26	Distal Splenorenal	21.8	18.8	−14	0.084	0.084
34	Distal Splenorenal	19.0	20.0	+5	0.021	0.021
37	Mesocaval	18.8	13.9	−27	0.042	0.0050

* According to Wilsoxon's *t* test (two-tailed analysis), *P* values for the differences in MRUS and in sensitivity to NH_4Cl in the nine subjects before and after shunt surgery were less than 0.05 and less than 0.01, respectively.

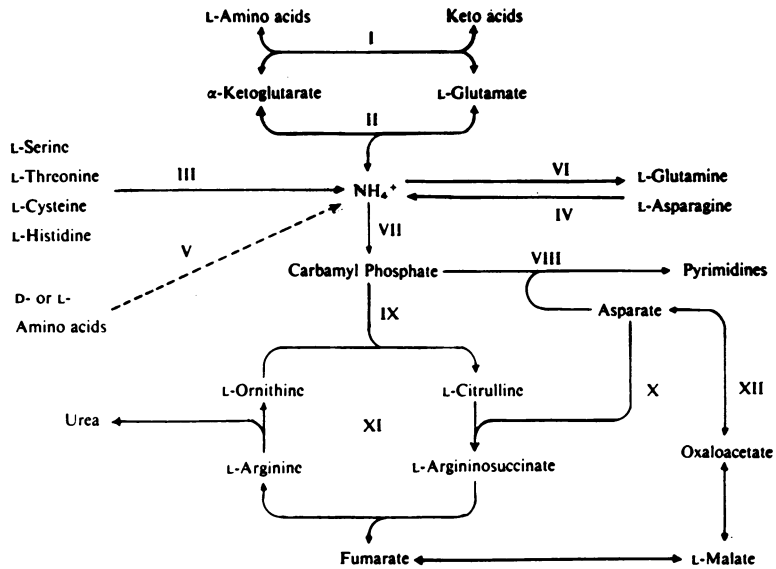

FIGURE 2 Summary of the general reactions involved in the nitrogen metabolism of the amino acids. Reprinted, with permission of the publishers, from reference 15, in which enzymatic mechanisms of reactions I–XII are discussed.

MRUS is correlated with certain clinical features in the cirrhotic patients. (*a*) When the patients eat 40–100 g protein daily, fasting blood or plasma levels of NH_3 and amino acids are inversely related to their MRUS. (*b*) Blood NH_3 concentration 2 h after ingestion of standard doses of NH_4Cl is inversely related to MRUS. Relationships (*a*) and (*b*) are to be expected if amino acids and NH_3 are the precursors and inter-

TABLE VI

*Effect of a Diet Containing 40, 60, 80, or 100 g Protein/Day for 10 Days on Blood Chemistry and Neurologic Status of Cirrhotic Patients of Groups I, II, III, IV, and V**

			40 g protein/day			60 g protein/day			80 g protein/day			100 g protein/day		
Group	Case no.	MRUS	NH_3	AAN	Encephalopathy	NH_3	AAN	Encephalopathy	NH_3	AAN	Encephalopathy	NH_3	AAN	Encephalopathy
		mg urea N/h/kg BW$^{3/4}$	*μg/100 ml*	*mg/100 ml*		*μg/100 ml*	*mg/100 ml*		*μg/100 ml*	*mg/100 ml*		*μg/100 ml*	*mg/100 ml*	
I	11	64.1							65	7.4	0	65	7.9	0
	12	56.1	45	6.1	0	61	7.5	0	50	6.4	0	48	7.0	0
	13	51.4							74	7.9	0	71	6.4	0
	14	56.3				56	7.5	0	83	7.8	0	53	7.0	0
I	15	45.4							105	7.5	0	96	7.1	0
	16S	46.6	70	6.2	0	73	7.4	0	81	7.8	0	102	8.6	0
III	17S	39.0	75	7.1	0	81	7.8	0	75	7.2	0	110	7.1	0
	18S	34.6	63	7.2	0	65	6.6	0	91	8.4	0	90	7.1	0
	23	30.1	71	7.3	0	95	7.5	0	83	6.7	0	153	9.2	+
	24	32.5	54	6.0	0	93	7.7	0	112	7.9	0	175	8.2	0
IV	21S	25.5	48	8.3	0	135	8.7	0	110	8.3	0	146	9.2	+
	23	29.7	61	7.9	0	128	8.7	0	180	8.3	+			
	26	21.8	73	8.4	0	170	9.8	+						
	27S	25.0	50	8.9	0	145	9.6	+						
V	40S	16.1	137	7.9	0	178	8.4	+						
	41S	13.2	115	7.5	0	160	8.3	+						
	42S	10.0	165	9.1	0	190	9.6	+						
	43S	9.5	135	8.5	0	184	8.9	+						
	44S	5.7	183	8.5	0	230	9.7	+						

* Blood NH_3 and plasma AAN values represent averages of those obtained on last 3 days of each period. Criteria for encephalopathy are described in the text. Cases were designated *S* after shunt surgery.

mediate product, and urea is the end-product, of a blocked metabolic sequence. (*c*) MRUS is significantly ($P < 0.05$) lower in cirrhotic patients with a history of encephalopathy (average ±SE, 15±1.5 mg urea N/kg $BW^{3/4}$/h) than in those without (average ±SE, 31±2.2). Only group V cirrhotics (MRUS < 20 mg urea N/h per kg $BW^{3/4}$) had experienced encephalopathy (Table IV). These findings are to be expected if one of the intermediaries of the restricted pathway, NH_3, is encephalopathic. (*d*) MRUS was further lowered in five of nine cirrhotic patients by shunt surgery (Table V). This reduction is to be expected if MRUS is influenced not only by activities of hepatic enzymes of amino acid catabolism and urea synthesis, but also by rate of delivery of substrate and fuel [8] to these enzyme systems. Why shunt surgery reduces MRUS in some patients but not in others is uncertain at present; the type of anastomosis (14) may be influential.

The present study suggests three ways in which measurement of MRUS may be useful in management of cirrhotic subjects. (*1*) Reduced ability to synthesize urea can be detected earlier in the natural history of cirrhosis than hyperammonemia, hyperaminoacidemia, and hepatic encephalopathy. The latter complications tend to occur when more than 70% of normal capacity has been lost (Tables IV and VI). (*2*) Table VI suggests that MRUS may predict how much dietary protein the cirrhotic patient can tolerate without developing hyperammonemia, hyperaminoacidemia, and encephalopathy. (*3*) Among nine patients studied before and after shunt, two were in group V preoperatively. One of these (case 37) was the only one of the nine to develop encephalopathy postoperatively (Table V). Perhaps measurement of MRUS before shunt surgery will identify patients at greater risk to postoperative encephalopathy; for such patients "selective shunts" (14) may be indicated.

Only cirrhotic patients without ascites and without impairment of renal function (Table IV) were tested. Either complication may reduce precision of the MRUS test: (*a*) Calculation of the amount of urea which accumulates in body water during experimental period 8–24 h assumes that urea space equals body water space as measured with ^3H_2O. This equivalence within ±5% has been demonstrated in the normal cat, dog, and man (9–12). In the ascitic patient, however, the two spaces may not be identical. Furthermore, in a patient with ascites kg $BW^{3/4}$ is unsuitable for adjusting dose of protein and MRUS to metabolic mass. (*b*) In subjects with normal renal function, less than 10% of urea synthesized during hours 8–24 of the MRUS experiment accumulates in body water, and over 70% is excreted in the urine. If kidney function is impaired, the former proportion will rise and the latter fall. Precision of the MRUS test will be reduced, since urinary urea excretion is measured directly, whereas accumulation of urea in body water is calculated as the product of two experimental variables (BUN and body water space).

[8] Two steps in the Krebs-Henseleit cycle require ATP.

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