Creatinine Metabolism in Humans with Decreased Renal Function: Creatinine Deficit

James D. Jones and Phillip C. Burnett

This study was designed to test the hypothesis that, in a patient with decreased renal function and an increased plasma creatinine concentration, a significant quantity of creatinine is excreted into the gut, as is true of urea and uric acids, and is metabolized by gut flora. [Methyl-14C]creatinine was given intravenously to five patients and [methyl-14C]creatinine or [carbonyl-14C]creatinine was given orally to five patients. Extracts of excreta, plasma, and urine were subjected to ion-exchange chromatography, with monitoring for 14C and for ninhydrin-positive material. Respiratory gases, collected in acid and base, were assayed for radioactivity. Blood specimens were obtained at intervals to furnish data on the decay of labeled creatinine in the body pool. The data show that there is a true creatinine deficit (15.9 to 65.7% of the creatinine formed is metabolized or excreted via extrarenal routes) in patients with decreased renal function. In these patients, creatinine is metabolized to CO2 and methylamine, presumably by the microflora of the gut. A significant portion of the carbonyl-labeled creatinine appeared in plasma in an unidentified compound.

Additional Keyphrases: chronic renal failure • extrarenal excretion of creatinine • isotope dilution technique • radiochemistry • "secretion" of creatinine into the gut • renal function • ion-exchange chromatography • creatinine metabolism • measurement of glomerular filtration rate

No direct evidence has been published to show that endogenously formed creatinine is metabolized in humans. The observed accumulation of creatinine in the serum, 30 mg or more per liter per day, in patients with acute oliguric renal failure is predictable from consideration of the excretion rates found in normal persons. In contrast, the expected rate of excretion plus excretion in patients with chronic renal failure is more frequently about half or less of the predicted values. We have termed this discrepancy "creatinine deficit." It has been observed by many investigators (1) and can be demonstrated readily in patients whose serum creatinine concentrations are greater than 4.5 mg/dl (2) or 6 mg/dl (3).

The apparent deficit has most frequently been attributed to decreased creatinine production or a decreased muscle mass. Attempts to implicate a decrease in muscle mass by Goldman (3) were unsuccessful, and he could not find more than 23 mg of creatinine per day in the feces of patients with increased serum creatinine concentrations. He concluded that an increased excretion as creatine in urine or excretion as creatinine in feces was excluded and that the decreased excretion in the urine was due to either a decreased rate of production or an undescribed alternative excretory pathway.

Creatinine has been assumed to be metabolically inert since the reports that creatinine labeled with 15N (4) or with methyl-14C (5) was not metabolically transformed into creatine and was excreted almost quantitatively as creatinine in the urine in animals with normal renal function.

Our initial efforts were based on the premise that creatinine most probably would be handled like urea and uric acid—that is, excreted into the gut and metabolized by gut flora—thereby giving a second route of clearance. About 25% of the urea (6) and 30 to 40% of the uric acid (7) made by normal man are cycled through the gut in this manner. The amount of creatinine available for such cycling would increase in patients with decreased renal function because serum

Mayo Clinic and Mayo Foundation, Rochester, Minn. 55901. Received May 27, 1974; accepted June 6, 1974.
creatinine concentrations increase severalfold in such patients. Furthermore, the gut frequently has been implicated as the source of abnormal compounds (“toxins”), and we anticipated that gut-originated metabolites of an endogenously formed substrate, from a metabolic conversion estimated to account for more than 500 mg/day, might be of physiologic and (or) clinical significance.

Before the experiments in humans to be described here, we had demonstrated that “creatininase” activity (the ability to degrade creatinine) could be induced in the gut flora of rats by feeding creatinine and that labeled methylamine, CO₂, sarcosine, and methylhydantoin were produced when [methyl-14C]creatinine was incubated with colon contents from such rats (8).

The experiments described here demonstrate by conventional isotope dilution methods that there is a creatinine deficit in patients with decreased renal function and that creatinine production exceeds the rate of accumulation plus excretion. Metabolites have been identified that implicate the flora of the gut.

**Methods**

*Patients.* We studied two anephric patients who were being maintained on chronic hemodialysis, six patients with relatively stable chronic renal failure as evidenced by little change in serum creatinine concentrations, and one patient with minimal renal function who was being maintained on dialysis for 8 h per week (Table 1). Patients 2 and 8 were not ambulatory when studied. The duration of the experiments varied and was determined by anticipated turnover time and availability of the patient for the procedure. The time of administration of labeled creatinine varied from 0700 to 1400 to avoid interfering with other parts of the patient’s schedule. No treatment was altered to accommodate the experiment or changed during the experiment.

Table 1. Description of Patients and Experimental Details

<table>
<thead>
<tr>
<th>Pt.</th>
<th>Age (yr); sex</th>
<th>Site of label &amp; route</th>
<th>Dose (μCi)</th>
<th>Body wt (kg) Initial</th>
<th>Final</th>
<th>Duration of experiment (h)</th>
<th>Disease</th>
<th>Diet restrictions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41; F</td>
<td>CH₃; i.v.</td>
<td>143.0</td>
<td>53.2</td>
<td>51.8</td>
<td>95.8</td>
<td>Chronic glomerulonephritis</td>
<td>20 g protein; Na restriction</td>
</tr>
<tr>
<td>2</td>
<td>70; F</td>
<td>CH₃; i.v.</td>
<td>157.9</td>
<td>67.7</td>
<td>69.3</td>
<td>63.5</td>
<td>Renal failure sec. to glomerulonephritis</td>
<td>20 g protein</td>
</tr>
<tr>
<td>3</td>
<td>57; F</td>
<td>CH₃; i.v.</td>
<td>130.8</td>
<td>54.1</td>
<td>53.9</td>
<td>89.7</td>
<td>Chronic renal failure sec. to bilat. cortical atrophy &amp; nephrocalcinosis</td>
<td>18 g protein; H₂O, Na, K restriction; dialysis 8 h/wk</td>
</tr>
<tr>
<td>4</td>
<td>19; M</td>
<td>CH₃; i.v.</td>
<td>143.0</td>
<td>60.1</td>
<td>62.3</td>
<td>86.3</td>
<td>Anephric, congenital urinary anomalies, solitary kidney, sigmoid conduit</td>
<td>50 g protein; regular dialysis, 9 h twice/wk</td>
</tr>
<tr>
<td>5</td>
<td>56; F</td>
<td>CH₃; i.v.</td>
<td>157.9</td>
<td>63.6</td>
<td>65.9</td>
<td>87.8</td>
<td>Anephric, chronic glomerulonephritis</td>
<td>40 g protein; 800 calories; dialysis, 8 h twice/wk</td>
</tr>
<tr>
<td>6</td>
<td>42; M</td>
<td>CH₃; oral</td>
<td>157.9</td>
<td>65.4</td>
<td></td>
<td>45.6</td>
<td>Chronic glomerulonephritis</td>
<td>30 g protein; 90 mmol Na</td>
</tr>
<tr>
<td>7</td>
<td>48; M</td>
<td>C=O; oral</td>
<td>148.6</td>
<td>80.7</td>
<td></td>
<td>56.1</td>
<td>Chronic renal failure sec. to glomerulonephritis</td>
<td>23 g protein</td>
</tr>
<tr>
<td>8</td>
<td>28; M</td>
<td>C=O; oral</td>
<td>140.0</td>
<td>82.4</td>
<td>82.5</td>
<td>70.8</td>
<td>Chronic membranous glomerulonephritis</td>
<td>50 g protein; 90 mmol Na</td>
</tr>
<tr>
<td>9</td>
<td>33; M</td>
<td>C=O; oral</td>
<td>157.9</td>
<td>82.3</td>
<td></td>
<td>217.0</td>
<td>Chronic renal failure, urinary tract infection</td>
<td>20 g protein; 2,400 calories; 90 mmol Na; H₂O, K unrestricted</td>
</tr>
<tr>
<td>10</td>
<td>22; M</td>
<td>C=O; oral</td>
<td>150.0</td>
<td>53.6</td>
<td>55.6</td>
<td>94.6</td>
<td>Chronic renal failure (sec. to glomerulonephritis or pyelonephritis), megacystitis with total unilateral ureterovesical reflux</td>
<td>25 g protein; no Na restriction; did not eat regularly; question of following diet</td>
</tr>
</tbody>
</table>

1 All subjects were patients of Mayo Clinic; they were selected after the purpose and design of the study had been fully explained and they had given informed consent.
Table 2. Creatinine Production and Metabolism

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Plasma creatinine, mg/dl</td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>15.30</td>
</tr>
<tr>
<td>Final</td>
<td>15.45</td>
</tr>
<tr>
<td>X</td>
<td>15.4</td>
</tr>
<tr>
<td>K (mg/h)</td>
<td>39.8</td>
</tr>
<tr>
<td>Slope (h⁻¹)</td>
<td>0.0042</td>
</tr>
<tr>
<td>Intercept</td>
<td>6.0654</td>
</tr>
<tr>
<td>S_pK</td>
<td>0.007</td>
</tr>
<tr>
<td>Creatinine space, liters</td>
<td></td>
</tr>
<tr>
<td>Extrapolated</td>
<td>26.73</td>
</tr>
<tr>
<td>²H₂O</td>
<td>25.36</td>
</tr>
<tr>
<td>Pool (mg)</td>
<td>4116</td>
</tr>
<tr>
<td>Production (mg/day)</td>
<td>955</td>
</tr>
<tr>
<td>Accumulation (mg/day)</td>
<td>0</td>
</tr>
<tr>
<td>Urinary excretion (mg/day)</td>
<td>531</td>
</tr>
</tbody>
</table>

* Studies on patients 8 and 9 were only to obtain material for identification of metabolites.

b Calculated asymptotic values.

c From plot of log (C*) vs. time. C* is plasma creatinine radioactivity, in dpm/liter.

Sampling techniques. At selected intervals after the radiolabeled creatinine was administered, blood specimens were obtained. Expired air was passed through vials containing standardized acid or base. The ¹⁴C content of these vials was determined by liquid-scintillation counting. No label was detected in the vials containing the acid. The counts retained in the base were assumed to be in CO₂. The recovery of the label in CO₂ was determined by assuming 0.15 mmol of CO₂ per kilogram of body weight per minute as the production rate (values obtained on patient 6 were 0.14 and 0.15).

Total collections of urine and feces were made from the time the labeled creatinine was administered until the experiment was completed. The feces were frozen until assay by placing them on solid CO₂ immediately after they were passed. Aliquots of blood, feces, and urine were counted in a liquid-scintillation counter. Sulfosalicylic acid extracts of the specimens were subjected to ion-exchange chromatography (8) with monitoring by liquid-scintillation counting and the ninhydrin reaction. The specific activity of creatinine was determined from the counts recovered from the column (internal standards were recovered quantitatively) and from the creatinine concentrations of the specimens as determined by reaction with alkaline picrate on an AutoAnalyzer (Technicon AutoAnalyzer; File N-11b; Technicon Instruments Corp., Tarrytown, N. Y. 10591). The creatinine concentrations in gastric contents and intestinal perfusates were also determined after preliminary isolation with Lloyd’s reagent.

Identification of metabolites was based on retention times on the ion-exchange columns and co-chromatography with known substances (8).

Calculations. Standard methods were used to calculate turnover and pool size. Creatinine space was calculated as water space (10). The size of the body pool of creatinine was determined by extrapolation, to zero time, of the isotope dilution curve in those patients who received the labeled creatinine intravenously (see Appendix). Body water was also determined with ²H₂O in four of these patients (11). The body pool size was determined only by the ²H₂O method in those patients who received the labeled creatinine orally. The concentration of creatinine in body water used in the calculations for the stable patients is given as X in Table 2.

Body weights changed negligibly and were not used in the calculations of creatinine pool size and turnover. These steps were taken for the following two reasons: (a) calculations with these values would be based on additional assumptions, and (b) the plot of log specific activity against time with “corrected” values did not yield values for creatinine production and deficit substantially different from those obtained without allowing for any change.

We calculated the rate of creatinine turnover with the assumptions that the rate of creatinine formation was constant, that creatinine is distributed in body water, and that physiologic variations were not important. These assumptions appear to be valid because plots of log₁₀ [¹⁴C]creatinine, C*, against time were linear. The labeled creatinine in the body pool
Table 3. Recovery of 14C From Renal Patients Given 14C-Labeled Creatinine

<table>
<thead>
<tr>
<th>Pt.</th>
<th>14C In</th>
<th>Body H2O</th>
<th>Urine</th>
<th>CO₂</th>
<th>Total</th>
<th>% of dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Creatinine</td>
<td>89 236</td>
<td>168 500</td>
<td>...</td>
<td>258 112</td>
<td>73.6</td>
</tr>
<tr>
<td></td>
<td>Metabolites</td>
<td>8826</td>
<td>3510</td>
<td>19 380</td>
<td>32 090</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>98 062</td>
<td>172 010</td>
<td>19 380</td>
<td>290 202</td>
<td>82.8</td>
</tr>
<tr>
<td>6</td>
<td>Creatinine</td>
<td>118 321</td>
<td>88 513</td>
<td>...</td>
<td>206 834</td>
<td>59.0</td>
</tr>
<tr>
<td></td>
<td>Metabolites</td>
<td>71 133</td>
<td>4 170</td>
<td>32 700</td>
<td>109 074</td>
<td>31.1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>189 454</td>
<td>92 683</td>
<td>32 700</td>
<td>315 908</td>
<td>90.1</td>
</tr>
<tr>
<td>5</td>
<td>Creatinine</td>
<td>205 514</td>
<td>702</td>
<td>...</td>
<td>206 216</td>
<td>58.8</td>
</tr>
<tr>
<td></td>
<td>Metabolites</td>
<td>17 924</td>
<td>4 552</td>
<td>22 379</td>
<td>44 855</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>223 438</td>
<td>5254</td>
<td>...</td>
<td>251 071</td>
<td>71.6</td>
</tr>
<tr>
<td>7</td>
<td>Creatinine</td>
<td>124 562</td>
<td>985</td>
<td>53 345</td>
<td>178 892</td>
<td>54.2</td>
</tr>
<tr>
<td></td>
<td>Metabolites</td>
<td>39 891</td>
<td>13 734</td>
<td>53 491</td>
<td>108 766</td>
<td>32.9</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>164 453</td>
<td>14 719</td>
<td>53 491</td>
<td>287 658</td>
<td>87.1</td>
</tr>
</tbody>
</table>

* Patients 2 and 5 received [methyl-14C]creatinine, 350.5 x 10^6 dpm, intravenously; patient 6 received the same dose and label by mouth; patient 7 received [carbonyl-14C]creatinine, 329.9 x 10^6 dpm, orally. Durations of experiments (in hours): patient 2, 63.5; 6, 45.6; 5, 87.8; and 7, 56.1.

had equilibrated by 6 h, and data obtained for earlier intervals were not used in calculating turnover rates. A minimum of four points was used. Calculation of the turnover rates in the patients being maintained on hemodialysis, and who therefore had changing pool sizes, involved additional steps; the details are given in the Appendix. The approach was based on the assumption of a simple one-compartment clearance and a constant production rate. Table 3 shows data, presented as a balance sheet, for four patients on recovery of label in body fluids and distribution of label between creatinine and metabolites. It has been assumed for the purpose of calculation of recovery, without documentation and probably incorrectly, that the plasma concentration directly reflects the distribution of all labeled metabolites in the entire body.

Results

**Curve of plasma disappearance.** The log₁₀ of the 14C (dpm) in creatinine was plotted against time, and the intercept and slope of the lines were determined by mean least squares (Table 2). The first sample that was used for these calculations was that obtained at least 6 h after isotope was administered, by which time the body pool had equilibrated, as evidenced by the linear decrease in log of specific activity with time from that time on (Figure 1).

**Pool size and creatinine deficit.** The calculation of the rate of creatinine production, K, in patients with an increasing plasma creatinine concentration requires determination of the plasma concentration at which rate of formation equals rate of clearance. This is the intercept, on the plasma concentration axis, of a plot of exp (- (a + b)t/V) against plasma creatinine concentration. In the two anephric patients this plot yielded a straight line (Figure 2). The calculation by manual integration gave similar values for rate of creatinine production: for patient 4, 1825 and 1752 mg/day; for patient 5, 1405 and 1407 mg/day.

In patient 3, who had residual renal function and was on restricted water intake, the function was curvilinear although a plot of plasma creatinine against time did show an asymptotic value of about 9.1 mg/dl (Figure 3). Therefore, the manual integration was
used to calculate rate of creatinine production. The quantity of creatinine excreted in the urine of this patient per unit time was not related to the plasma concentration measured at that time. During this interval, the plasma creatinine concentration increased from 6.2 to 9.1 mg/dl with no apparent change in rate of creatinine excretion. This indicates that this patient, with plasma creatinine concentrations less than 10 mg/dl, was excreting creatinine in the urine by a mechanism other than filtration and which was not concentration-dependent. This more complex situation may account for the nonlinearity of the plot, and clarification must await studies in additional patients of this type.

In the patients not on dialysis, the creatinine pool varied from 1.7 to 6.4 g. The rate of production varied from 32 to 73 mg/h, and the rate of metabolism from 251 to 715 mg/day. The data indicate that 16 to 66% of the creatinine formed in this group of renal patients is continually being metabolized.

Estimation of the rate of metabolism from the appearance of metabolites is not possible because we do not know the pathway(s) of metabolism or the specific activity of identified metabolites except CO2.

Recovery of 14C in creatinine and metabolites. By ion-exchange chromatography of specimens from patients given [methyl-14C]creatinine, 14C was identified in two acidic compounds, in a compound appearing 5 to 10 ml after urea, in one with or slightly after sarcosine, in methylamine, and in trace amounts in an unidentified compound; CO2 in the expired air also contained 14C. 14C was not present in methylguanidine. When [carbonyl-14C]creatinine was used, all of these metabolites except methylamine contained 14C.

The presence of an as-yet-unidentified metabolite of creatinine is suggested by the following facts. When [methyl-14C]creatinine was administered to patients, recovery of 14C in creatinine and metabolites from the ion-exchange column was quantitative at the early intervals. At the last sampling times, the lowest recovery observed was 89% in the plasma of patient 3, 89.7 h after she received the labeled creatinine. In contrast, 14C was poorly accounted for analytically in plasma specimens from patients given [carbonyl-14C]creatinine; only 75 to 84% of the 14C was recovered from the first plasma specimens from patients 7, 8, and 10. In the last specimens from these patients, the recovery was 66 to 76%. Dialysates obtained at the first therapeutic hemodialysis at the termination of the experiments with patients 8, 9, and 10 showed the same phenomenon. As in the chromatography of other specimens, changing the conditions to those that would elute the very basic polyamines did not yield additional 14C. We now believe that the compound(s) responsible is not CO2 and is labile both in stored plasma and dialysates and in sulfosalicylic acid extracts of these fluids. The identity of this compound(s) is currently under investigation.

At the end of the experimental period, the recoveries of 14C from urine, excreta, CO2, and body water (calculated from plasma concentrations) ranged from 72 to 90%. Estimates, based on the rate of excretion during the experiments, of the quantity of 14C remaining in the gastrointestinal tract at the termination of the experiments would increase these recoveries by less than 1%. This inability to account for a significant portion of the administered 14C is interpreted as another indication of a retained and as yet undetermined metabolite(s).

Kinetics of creatinine metabolism. That the labeled creatinine is metabolized in the gut may be surmised from the appearance, with time, of labeled CO2 and metabolites in the body fluids. Labeled metabolites can be detected in the first plasma and urine specimens obtained, whether the creatinine is labeled in the methyl or carbonyl group and regardless of the route of administration.

The CO2 expiration curves (Figures 4 and 5) reflect the final product not only from the intestinal microflora but also from metabolism, by the patient, of the metabolites formed from creatinine in the intestinal tract. The initial rapid evolution of 14CO2 after oral administration of labeled creatinine (patients 6, 7, and 8 in Figure 4) is interpreted to indicate metabolism of a bolus of unabsorbed creatinine in the gut. The later plateau, which is superimposable on the 14CO2 curve from the patients receiving the labeled creatinine intravenously (patients 1 and 2 in Figure 4), is interpreted as representing the metabolism of bacterial metabolites by the patient as well as the CO2 produced by the flora of the gut. The shapes of the individual curves in Figure 5 appear to be similar after the initial few hours, the quantity of CO2 evolved being determined by the patient's residual renal function. The proportion of 14CO2 produced by the intestinal microflora directly has not been estimated.

Table 4 shows that creatinine is "secreted" into the gut. We obtained these data on subjects who had no
renal impairment. The values for gastric contents were obtained from aspirates from the resting stomach. The concentration of creatinine in the solutions perfused through the intestinal tract appeared to increase from the upper to the lower tract. In similar situations the concentration of urea in the gut lumen would be one-half to two-thirds that in the blood.2 These values are given only to illustrate that endogenous creatine does get into the gut. A relationship of concentration to existing serum creatinine concentrations was not apparent, probably because of the narrow range of serum creatinine concentrations—that is, at these concentrations, the values obtained probably reflect collection nonequilibrium conditions more than concentration gradients.

Discussion

The evidence presented here indicates that a variable portion, 15.9 to 65.7%, of the creatinine formed endogenously in persons with decreased renal function is metabolized. The following evidence supports this conclusion: (a) the estimated rate of creatinine production exceeded the measured rate of creatinine excretion plus accumulation in body fluids; (b) radio-

diolabeled creatinine accounted for in urine plus that calculated to remain in body water after administration was incomplete; and (c) the 14C administered as [14C]creatinine was found in metabolites in plasma and urine. Thus, an explanation is available for the absolute decrease in urinary excretion of creatinine with increasing serum creatinine concentration observed by many and described graphically by Enger and Blegen in 1964 (2).

Efforts to implicate enzymes in the formation of creatinine from creatine and creatine phosphate have been unsuccessful (12). These essentially irreversible reactions are now accepted as nonenzymatic. Creatinine formation would be decreased by a decrease in muscle mass (that is, in the precursors creatine and creatine phosphate). However, this deficit could not be related to a decrease in muscle mass (3). These facts support the concept that the creatinine deficit is due to metabolism of a significant portion of the creatinine synthesized.

Evidence for a decreased rate of creatine synthesis, and hence of creatinine synthesis, in nephrectomized rats has been presented by Horner (13) and Goldman and Moss (14,15). Those experiments proved conclusively that creatine is synthesized in extrarenal tissues. A lower extent of incorporation of a labeled precursor, [14C]glycine, into muscle creatine was interpreted as indicating a decreased rate of synthesis of creatine. Using the same experimental plan, they (15) observed that creatinine loading by intraperitoneal injection also, but less effectively, decreased incorporation of [14C]glycine and [14C]guanidinoacetate into muscle creatine in both nephrectomized and control rats. However, the conclusion that the rate of synthesis can be determined by this approach may be incorrect because (a) plasma glycine (16) and guanidineacetate (17) have been reported to be increased

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2 Personal communication, S. F. Phillips.
in renal failure and, if such were true in these animals, incorporation from an increased precursor pool would cause the observed change without alteration of rate of formation of creatine, and (b) these animals did not show a comparable decrease, but maintained muscle creatine concentrations comparable to those of controls.

The accurate estimation of the rate of production of creatinine depends on an accurate determination of the body pool. Although it is well documented (10) that creatinine is distributed in body water, special phases of body fluids exist with probably different creatinine concentrations. These differences are believed to be too small to introduce significant error into the estimation of the body pool of creatinine. The body water space independently determined by \( ^2\text{H}_2\text{O} \) closely approximated the value obtained by extrapolation of plasma specific activity to the time of injection.

The calculated creatinine spaces as percentage of body weight were 55.3 to 67.5 for the men and 37.4 to 51.4 for the women (in patients 2 and 5 they were 37.4 and 45.8%; these patients had more body fat). Determination of body water by \( ^2\text{H}_2\text{O} \) has a precision of \( \pm 2\% \), as does the determination by \( ^{14}\text{C} \) creatinine distribution extrapolated to zero time.

Because clearance by nonactive processes is concentration-dependent, the concentration of creatinine in plasma is a direct reflection of clearance(s). As renal function decreases, the plasma concentration will increase until the clearance equals the rate of formation. When renal function becomes severely decreased or, as in the anephric patient, other routes of excretion become more important, this plateauing of plasma creatinine concentration does occur; use was made of this fact in calculating the turnover in those patients with changing plasma creatinine concentrations.

Until detailed data are obtained on more patients in additional clinical states and under different treatments, the factors affecting this final concentration will remain unknown. It is reasonable to anticipate that some of the variability in urinary excretion of creatinine in normal persons is caused by a similar phenomenon. More nearly accurate estimations of the rate of formation in normal individuals and alterations in metabolism by imposing intestinal bacteriostasis should clarify this point and settle the question of absolute decrease in creatinine formation in patients with renal disease.

Among the metabolites of labeled creatinine separated by ion-exchange chromatography, methylamine was identified in plasma and urine. Two acidic metabolites appeared consistently in the chromatograms of urine and plasma. Other regions of the chromatogram contained small amounts of radioactivity and are being studied. The quantities of \( ^{14}\text{C} \) in these metabolites varied from patient to patient; for example, patient 3 had more label in metabolites and a much more complex pattern of radioactivity distribution in the chromatogram than did the other patients, including those (patients 4 and 5) on more frequent dialysis. Some of these metabolites may reflect re-incorporation of \( ^{14}\text{C} \) into known compounds not unique to creatinine metabolism.

In addition, a major unknown metabolite of [carbonyl-\( ^{14}\text{C} \) creatinine was found in these patients, but the experimental design complicated our efforts to identify the compound(s). First, because we obtained only the minimal amount of blood needed for the objective of the experiment, only minute quantities of plasma were stored. The specimens were counted and filtrates were prepared when they were obtained; chromatography, a slow process, was performed some time later. The noncreatinine \( ^{14}\text{C} \) may be lost to the container or to air on storage. If the unknown survived storage, it may be lost on the column. The \( \text{CO}_2 \) released from such specimens upon addition of acid was not radioactive. On the positive side, we can demonstrate the presence of an unknown compound(s) by difference in radioactivity in these specimens. The compound(s) was present in dialysate as well as plasma from patients who received [carbonyl-\( ^{14}\text{C} \) creatinine. Identification is being actively pursued. It is interesting to speculate that, because the quantity of labeled material is significant and is maintained at a relatively constant level throughout the experimental period, the compound(s), when identified, will be present in a relatively high concentration.

The assumption that all metabolites will appear in plasma is unwise. The presence of creatinine in sweat has been demonstrated by Consolazio et al. (18); although we did not evaluate creatinine loss by this route or by insensible perspiration, our patients were not physically active and uremic patients sweat very little. The quantity of the creatinine shown under "Metabolism" in Table 2 would include any lost by this route. Also unwise is the estimation of the total quantity of metabolites in the body pool by assuming that the distribution in plasma is similar to that in the body water, especially in light of the fact that only 72 to 90% of the label could be accounted for at the termination of the experiments. Use of creatinine labeled in other atoms should clarify this point and also elucidate the metabolism of the remaining portion of the creatinine molecule.

It is easy to see why it was assumed that creatinine was not metabolized in man. Maw (19) showed that he could recover almost quantitatively the creatinine from rats that received an oral dose of 30 to 60 mg and that the gut flora of the rat did not metabolize creatinine. Goldman (3) could not find significant quantities of creatinine in human feces. However, 25% of an oral dose of creatinine was shown to survive passage through the small intestine (20). Apparently, creatininase can be induced in the gut of patients as a side-effect of chronically decreased renal function. Our previous work indicated that the enzymatic activity could be induced in the microflora of
the gut of rats by feeding creatinine, although it was absent in preparations from rats on controlled diets (8). The demonstration here that creatinine actually does get into the gut allows creatinine to be added to the list of compounds that are presented to the flora of the gastrointestinal tract for metabolism. Therefore, creatinine metabolism is not an exception to a common phenomenon.

The specific activity of the metabolites was not determined, so we have not assessed the quantity of creatinine metabolized through a pathway(s) suggested by the structures of the compounds isolated. That the conditions of the in vitro incubations of gut contents (8) can alter the pathway, as evidenced by alteration in the ratios of products, indicates that there is much more to be learned about this qualitatively as well as quantitatively.

Currently it is not possible to assess the effect of metabolism of creatinine on a patient with decreased renal function. If we do not find a physiologically active metabolite that exerts an adverse effect, the overall effect will be to salvage nitrogen. This appears to be advantageous to the individual who can adapt to the disease and the diet.

That, in addition to excretion in the urine, there exists another major route of creatinine excretion that is variable explains the poor correlation observed between serum creatinine values and urinary creatinine clearances in some patients.

Clinical implications. The enteric cycling of creatinine and its bacterial metabolites is comparable to that demonstrated for urea (6, 21) and uric acid (7). There are two clinical implications.

First, the description of a second route of excretion of creatinine has created a dilemma regarding the usefulness of serum or plasma creatinine concentration as a measure of glomerular filtration rate. This relationship must be clarified to allow its use in identifying and monitoring patients with decreased renal function. Another, more reliable and easily measured index of glomerular filtration rate must be found.

Second, the metabolites of a compound, produced endogenously in amounts up to 643 mg/day, could have significant biochemical importance in a patient who cannot excrete them, as a consequence of decreased renal function.

We acknowledge the clinical support and valuable suggestions of Drs. W. J. Johnson, C. F. Anderson, S. F. Phillips, H. W. Wahner, and Kenneth Offord, and the technical assistance of Mrs. Patricia E. Lenton.

This investigation was supported in part by Research Contract 72-2206 from the NIH, USPHS.

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Appendix

The calculations for the rate of production of creatinine are based on the assumption of a one-compartment model.

Pool constant

- \( C_0 \) = initial concentration of creatinine in body water (mg/liter)
- \( V \) = volume of body water (liters)
- \( K \) = creatinine production rate (mg/h)
- \( a \) = urinary clearance rate (liters/h)
- \( b \) = other clearances (liters/h)
- \( A \) = plasma creatinine (mg/liter)
- \( C^* \) = plasma creatinine activity (dpm/liter)
- \( * \) = activity injected at time \( t = 0 \) (dpm)

During the experiment, at times \( t \), we measure \( A \), \( C^* \), and the clearance of creatinine in urine. In order to determine \( (a + b) \), \( V \), and \( K \):

\[
\log(C^*) = \log(*/V) - \left(\frac{a + b}{V}\right) \cdot \frac{t}{2.303}
\]

Hence, if one plots \( \log(C^*) \) vs. \( t \), the \( y \) intercept is \( \log(*/V) \), and the slope (change in \( \log(C^*) \) per unit time) will yield clearance values. Moreover, the constant creatinine pool size implies balanced production and clearance, hence:

\[
V = (*)/\text{antilog of } y \text{ intercept}
\]

\( (a + b) = V \cdot \text{slope} \cdot 2.303 \), and

\[
K = (a + b) \cdot A
\]

Pool increasing. When plasma creatinine is increasing during the observations, one can determine \( (a + b) \) and \( V \) as above, but one must adopt a different approach to calculating \( K \). Under the assumptions of constant clearances and constant production, plasma creatinine will vary with time as:

\[
A(t) = [C_o - K/(a + b)] \exp\left(-\frac{(a + b)}{V} \cdot t \right) + \frac{K}{(a + b)}
\]

Note that, from the earlier computations, one has a value for the factor \( (a + b)/V \) (specifically, it is 2.303 X slope of earlier plot). Hence, one can convert the abscissa values from time, \( t \), to a new form:

\[
q = \exp\left(-\frac{(a + b)}{V} \cdot t \right)
\]
If one now plots $A$ vs. $q$ and takes a best-fit straight line, the intercept on the $A$ axis will be $K/(a + b)$. This intercept is the asymptotic value for serum creatinine under the single-compartment model. Thus, $K = (a + b)A$ intercept. Note that the slope of the line on the $A$ vs. $q$ plot is, slope $= C_0 - K/(a + b)$. This can be used with the previously derived values to compute $C_0$ as a check on the reliability of the method.

References