American Industrial Hygiene Association Journal

ISSN: 0002-8894 (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/aiha20

INTERPRETATION OF URINE RESULTS USED TO ASSESS CHEMICAL EXPOSURE WITH EMPHASIS ON CREATININE ADJUSTMENTS: A REVIEW

Mark Frederick Boeniger , Larry K. Lowry & Jon Rosenberg

To cite this article: Mark Frederick Boeniger , Larry K. Lowry & Jon Rosenberg (1993) INTERPRETATION OF URINE RESULTS USED TO ASSESS CHEMICAL EXPOSURE WITH EMPHASIS ON CREATININE ADJUSTMENTS: A REVIEW, American Industrial Hygiene Association Journal, 54:10, 615-627, DOI: 10.1080/15298669391355134

To link to this article: https://doi.org/10.1080/15298669391355134

INTERPRETATION OF URINE RESULTS USED TO ASSESS CHEMICAL EXPOSURE WITH EMPHASIS ON CREATININE ADJUSTMENTS: A REVIEW

Mark Frederick Boeniger[a]
Larry K. Lowry[b]
Jon Rosenberg[c]

[a]National Institute for Occupational Safety and Health 4676 Columbia Pkwy, Cincinnati, OH 45226; [b]Midwest Research Institute 425 Volker Boulevard, Kansas City, MO 64110; [c]California State Department of Health Services, 2151 Berkeley Way, Berkely CA 94701

This paper reviews the process of elimination of creatinine (CRE), and the limitations presented when using it to express urine concentrations. This literature review leads to three conclusions: (1) CRE excretion is subject to wide fluctuations due to specific internal and external factors; (2) the use of CRE to correct chemical concentrations in urine will not necessarily improve the correlation to the exposure dose for all chemicals (it may, in fact, worsen the result); and (3) other means of expressing urine concentration may offer greater accuracy towards estimating individually absorbed dose.

The simplest means of expressing the elimination of materials in urine is mass per unit volume (i.e., unadjusted concentration). However, urine concentration may fluctuate due to many external and internal factors that are not related to exposure. It is desired to minimize the influences of these factors that may confound the interpretation of exposure.

Different means of expressing urine concentration have been used to improve the correlation of exposure to urine concentration. The use of creatinine (CRE) is one means of expression. Creatinine adjustment was first attributed to Hill in 1953, who normalized the analyses of aniline in urine.[1] Since then, it has become increasingly popular in the industrial hygiene field. However, without knowledge and appreciation of what is presently known about CRE excretion, its use to adjust urine sample concentration may be unjustified and lead to inappropriate conclusions.

This paper reviews the process of renal elimination of creatinine, external and internal factors affecting creatinine elimination, limitations of creatinine when used to adjust for urine dilution, and alternative methods for expressing the urine concentration of foreign substances.

DESCRIPTION OF RENAL ELIMINATION

In addition to the many important functions that the kidneys play in controlling the concentration of most of the constituents of body fluids, they provide the body with a means of eliminating waste products of bodily metabolism, i.e., low molecular weight solutes. Renal elimination of substances can be accomplished by filtration, active secretion, and passive diffusion.

The primary functional structure of the kidney is the nephron, of which there are about 2 million in both human kidneys (Figure 1). Blood flow from an afferent arteriole enters the glomerulus, which is a network of up to 50 parallel branching capillaries that are 100–500 times more permeable than the usual capillary. Compounds like inulin, with a molecular weight (MW) of 5200, are as freely permeable as water, whereas a compound with a MW of 30 000 is about half as permeable, and albumin, which is the smallest plasma protein (MW = 69 000) permeates to the amount of 0.5%. Around the glomerulus is a surrounding structure called Bowman's capsule, through which the filtrate is collected. The filtrate continues on into a proximal tubule and distal tubule until it reaches a collecting duct that leads to the ureters and urinary bladder. A branching network of efferent arterioles leave the glomerulus and are in close association with the tubules where substances may be exchanged by active secretion or passive diffusion across the membranes in either direction.

Filtration

The afferent arteriole leading into the glomerulus is larger than the efferent arteriole, causing a net filtration pressure of about 24 mmHg. This pressure forces fluid and small solutes from the plasma across the glomerular membrane

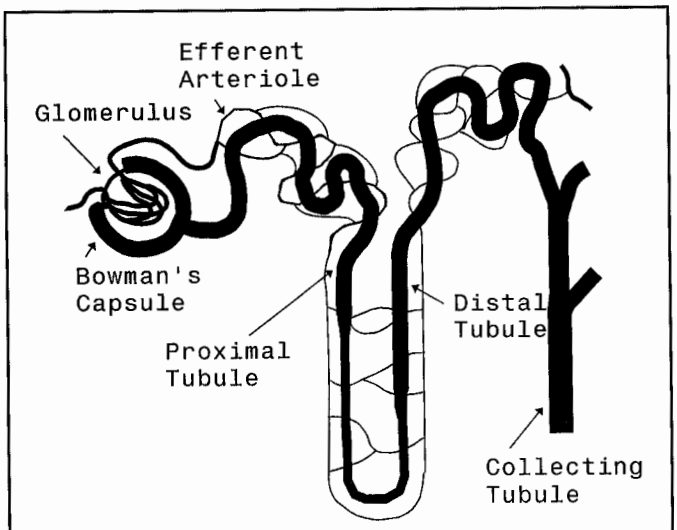

FIGURE 1. The kidney nephron

and into the capsule. In the average healthy individual, the glomerular filtrate formation rate is about 125 mL/min. This constitutes only a fraction of the renal blood-flow rate that averages about 1200 mL/min.[2] Any fluid or compound that is not reabsorbed across the renal tubules and returned to the blood plasma is passed into a collecting duct as urine.

Active Secretion

Actively secreted substances are actively transported, primarily through the proximal tubular lumen, against an electrochemical gradient. This carrier-mediated transport can be in either direction across the tubules, i.e., it can either add to or remove substances from the urine filtrate. Since the renal blood flow is greater than the glomerular filtrate volume, the proportion of substance presented to the kidney for active secretion of plasma substances can be significant. Inorganic potassium, hydrogen ions, and mercury are secreted. Because of the negative charge on the glomerulus basement membrane, most organic bases must be secreted. Organic compounds eliminated by active secretion include those that can be conjugated by the liver in the form of hippurates, glucuronides, and sulfates. Examples include penicillin, uric acid, certain sulfonamides, the dye phenol red, toluric acids, and p-aminohippuric acid. As the plasma concentration of these substances increases, the rate of secretion also increases up to a point when the enzyme binding sites become saturated, and no further increase can occur. When substances are eliminated by secretion, one would expect the rate of elimination to be generally independent of urine flow. Also, the concentration in urine over time would vary inversely in proportion to the volume of urine (Figure 2a-b). For compounds excreted in this way, urine flow will affect the concentration, but not the mass, of analyte excreted over time.

Passive Diffusion

In addition to filtration and active secretion, soluble substances may passively diffuse through the tubule or be passively reabsorbed back into the plasma. Equilibrium kinetics

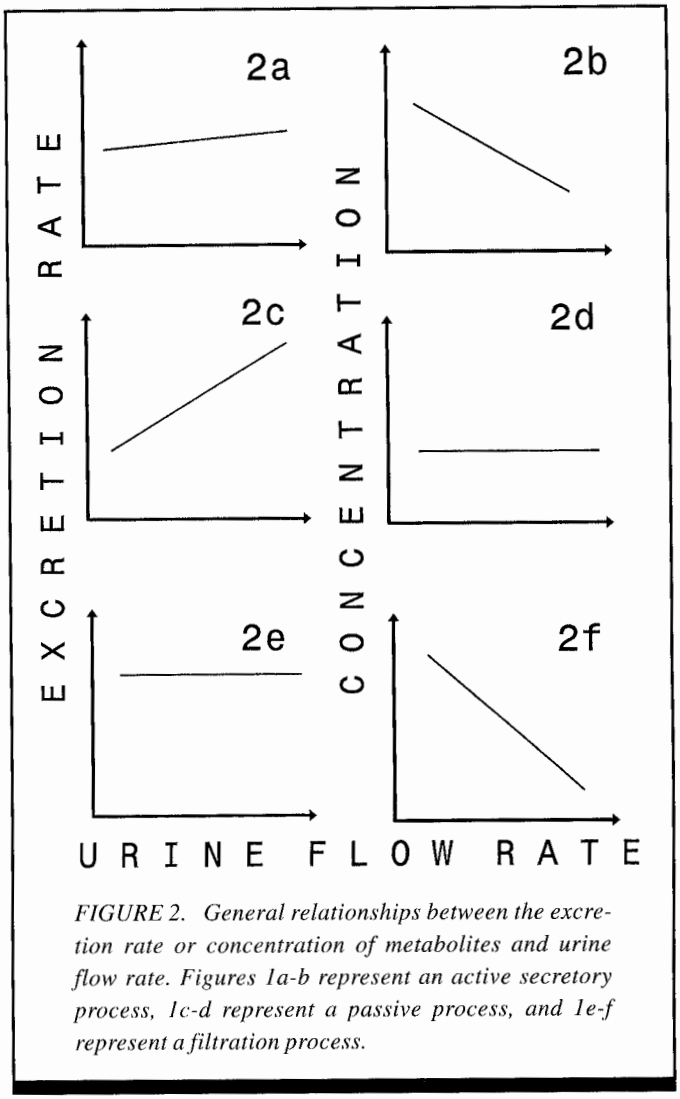

FIGURE 2. General relationships between the excretion rate or concentration of metabolites and urine flow rate. Figures 1a-b represent an active secretory process, 1c-d represent a passive process, and 1e-f represent a filtration process.

dictate the rates of these events, which are determined by the concentration differences of the chemical in solution, and the ability of the chemical to diffuse across the capillary/tubular boundary. Where passive diffusion plays a major role in renal elimination, the rate of elimination of the chemical from blood by the renal route is directly proportional to body hydration (diuresis) and, therefore, the rate of fluid passage from the kidney to the urinary bladder (Figure 2c). At the same time, the concentration of the chemical in urine is generally expected to be less dependent on the urine output, since the diffusion process is determined by the equilibration of partial pressures in urine and plasma (Figure 2d). This means of clearance occurs for such important industrial compounds as toluene,[3-4] methanol,[5] styrene as mandelic acid,[3,4,6] methyl mercury,[7] elemental lead,[8] and nitrous oxide.[9] Passive reabsorption of polar compounds and ions through the tubule is usually minimal, as is the case with 2-thiothiazolidine-4-carboxylic acid (after exposure to carbon disulfide) and with elemental mercury.[10-11] The elimination of ionic compounds is strongly influenced by changes in urinary pH, which is somewhat dependent on the rate of urine flow. Thus, many weak acids and bases present in plasma (pH $\approx$ 7.4) in their nonionized form are lipid soluble, and

diffuse readily across the cell membranes. Upon reaching the tubule, these weak acids and bases may be ionized, trapped in the tubule, and excreted if the urine pH (range 4.5 to 8) becomes too acidic (for bases) or too alkaline (for acids).

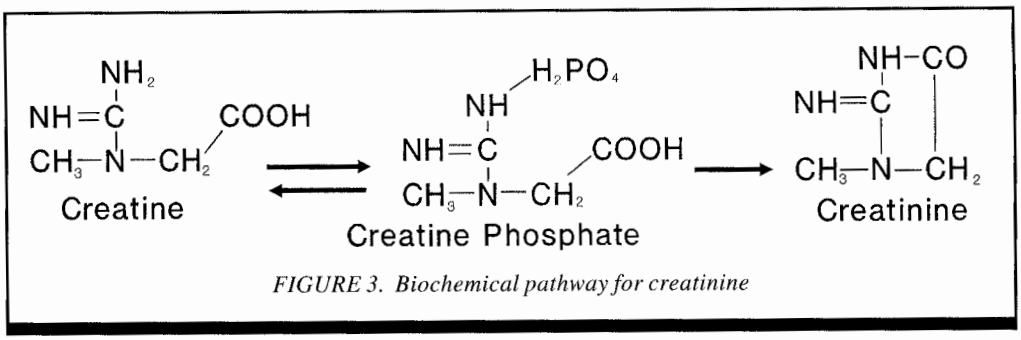

FIGURE 3. Biochemical pathway for creatinine

The mechanism of excretion of a substance can be discerned if the ratio of the plasma and urine concentration of the substance of interest can be compared to the clearance value of a highly filterable substance like CRE or inulin.[12] If the urine-to-plasma concentration ratio for the substance of interest is much larger than the reference value for the glomerular filtration marker, then an active secretory process is probably involved. Ratios substantially below the reference value indicate active tubular reabsorption of the substance. Concentration-dependent reabsorption also occurs by passive diffusion of non-ionic lipid soluble materials. Assuming the filtration marker to have a value of 100, aliphatic hydrocarbons have typical urine-to-plasma concentration ratios of 0.07–0.09, indicating substantial passive tubular reabsorption, while conjugated phenols have a value of 300, indicating substantial active secretion.[13]

RENAL ELIMINATION OF CREATININE

CRE is a normal endogenous end-product of human metabolism. It is a by-product that results from the formation and decomposition of creatine and creatine phosphate, which provide an essential link in the transformation of chemical energy into muscular work.[14] While most creatine is produced in the liver, approximately 94–98% of total body creatine ends up being actively accumulated within skeletal muscle (i.e., lean body mass).[14] In healthy persons, CRE is formed by an essentially irreversible, apparently nonenzymatic mechanism involving creatine dehydration, and CRE is cleared from the blood through the kidney.[14–16] Because almost all of the creatine/creatine phosphate is present in skeletal muscle, the amount of CRE eliminated from the body is generally related to the lean body mass of the individual. Among healthy male subjects, the creatine turnover rate is about 1.6–1.7% per day.[17] The biochemical pathway for the biotransformation of creatine and CRE is shown in Figure 3.

Since CRE is a low molecular weight, nonprotein bound nonelectrolyte, its passage or clearance from the blood into the kidney tubule is accomplished primarily through glomerular filtration and is theoretically proportional to the CRE concentration in blood. CRE is not re-absorbed by the renal tubules. If the rate of elimination of CRE is normally independent of urine flow (Figure 2e) its concentration in urine should be inversely related (Figure 2f).[18,19]

Renal plasma clearance is the ratio between the urinary excretion rate and plasma concentration, and is equal to the volume of plasma completely cleared in a given time. The mathematical expression for renal clearance is given as follows:

$$V_p = \frac{V_u \times C_u}{C_p} \quad (1)$$

where C_p and C_u denote concentrations of a substance (e.g., creatinine in plasma and urine, respectively), V_u is urine output per time unit, and V_p represents the renal clearance.

Under normal conditions, the clearance of CRE should parallel the glomerular filtration rate (about 125 mL/min). Glomerular filtration rate varies little with hydration.[20] However, the amount of CRE passed by secretion of a healthy male may be about 14% or more of the total, and the rate of secretion may vary significantly from one person to another.[21–22] The amount of secretion can be especially high in cases of low glomerular filtration and increases with plasma concentration.[23–24] Therefore, the calculated CRE clearance rate is not necessarily a true representation of glomerular filtration and presents a special concern for those using this index to make clinical determinations of renal sufficiency.

The above background provides information on renal clearance of CRE and other substances that is needed to interpret urine samples containing foreign substances (xenobiotics). One might conclude that CRE would have its greatest utility when used to adjust the concentration of a xenobiotic that is eliminated primarily through filtration and is not reabsorbed by the tubules in a way that is dependent on hydration. However, the influence of various factors, which will be discussed later, further limits the accuracy of such adjustments.

ANALYTICAL METHOD FOR DETERMINING CRE IN URINE

CRE determination in urine can be performed by several methods.[25] The majority of clinical analyses are most commonly performed using an automated colorimetric determination based on a modified Jaffe reaction.[26–28]

The Jaffe reaction, when used to determine CRE in blood, is subject to positive interferences from uric acid, protein, glucose, fructose, acetone, pyruvic acid, acetoacetate, and ascorbic acid, amounting to about a 20% overestimate in the result.[25] Normally, these interferences are not present in the urine. In one study in which the urine CRE

TABLE I. Factors Affecting Creatinine Elimination

Factor	Maximum Effect Range	References
Endogenous Factors		
Gender	≥ 10%	50, 51
Age	⇓ 15–25%[A]	40, 49
Muscularity	~100%	41, 42, 70
Diurnal	⇑ 60%[B]	18, 30, 36
Disease	varies	14, 16, 23, 53–56, 60
Pregnancy	⇑ 50%[C]	31, 63
Exogenous Factors		
Diet	≥ 50%[D]	45, 64, 69, 71–73
Urine Flow	≥ 50%[E]	77–83
Physical Activity	⇑⇓ 30–50%[F]	19, 34, 86–88
Medical Drugs	probably minimal	90
Tobacco	minimal	91
Incomplete Voiding	≤ 1%	94
Total Intraday	≥ 200%	30

[A]decreases after age 20
[B]compared to basal nighttime rate
[C]when pregnant
[D]with high meat diet
[E]mainly at urine flow < 1 mL/min
[F]decreases immediately followed by later increase

concentration was determined in over 100 individuals by both the Jaffe method and a true CRE method, no difference in the results occurred.[24]

Estimates of the analytical precision associated with the determination of CRE in urine have been reported. The coefficient of variation on replicate samples, using the automated Jaffe method, is between 2 to 3% on same day samples and about 4% on replications made on different days.[28–30]

In the literature, the accuracy of CRE analysis has received less attention than has precision. In one recently published account of a comparison among 11 laboratories that performed analysis of aliquots of a single urine specimen, there was substantial disagreement between laboratories.[31] The reported CRE concentrations ranged from 73 to 120 mg/dL (mean, 102). The most extreme values were reported by laboratories using identical instrumentation. Good laboratory practice requires the calibration of instrumentation using known "calibrators" obtained from a reputable clinical supplier. In order to document reliable quality data, quality control samples with assayed CRE concentrations must be included in each run. Several good reviews of analytical methods used to determine CRE can be found.[25,32]

FACTORS INFLUENCING ELIMINATION OF CREATININE IN URINE

Although CRE excretion has been assumed by some to be closely related to an individual's lean body mass, and both deviate little over the short term, the inter-day variability of CRE elimination is actually quite large, according to some reports.[33] The individual day-to-day range in differences between the lowest and highest 24-hour urine elimination increases with the number of days monitored. This was demonstrated in a report in which 8 healthy individuals collected an average of fifty-three 24-hour urines during a 6 to 10 month period of time. The difference between the high and low daily amount of CRE eliminated for each of these individuals over the collection period ranged from 63 to 244% (average = 107%).[34] Many additional studies confirm the large intra-individual variability in CRE elimination. A summary paper of 10 independent studies reported a coefficient of variation (CV) in the 24-hour CRE excretion to range from about 3% to about 20% in different subjects, with an average CV of about 10%.[30] A CV of 10% corresponds to a 95% confidence interval of ± 20% around the mean. The ratio of the top of this confidence interval divided by the bottom of the confidence interval is 1.5. Referring to the worst case (i.e., 20% CV), the CV corresponds to a 2.3-fold difference between the lowest and highest values.

The intra-day variation in partial day samples has been shown to be two to three times larger than the 24-hour inter-day variation. The group intra-day mean coefficients of variation, as reported in several independent reports, shows the average CV to range from a minimum of about 6% to about 30%.[30] In the latter case of a 30% CV, the upper confidence interval values are four times greater than the lower values for creatinine.

There are both internal and external influences that can affect the extent that CRE is found in the urine. These factors and the maximum magnitude of their influence are summarized in Table I.

Internal Factors

Many internal factors have been identified as influencing the elimination of creatinine in urine. Intra-personal biological variation is attributed to normal diurnal cycles. Interpersonal biological variation has been attributed to differences in body mass, age, sex, and health.

Diurnal Variations

The extent of diurnal or circadian variation in the urinary and plasma CRE concentration has been well documented. One group reported that the average plasma CRE concentration for a group of four adults increased from 0.61 mg/dL at 9 a.m. to 0.83 mg/dL at 3 p.m.[18] The average urinary CRE

elimination rate varied similarly, increasing about 35% in the first three hours after rising. The diurnal pattern that most often appears is such that the overnight and early morning urine samples contain less CRE than the late afternoon samples and are much less prone to inter-day variations.[18,30,35–37] In five different studies, the difference between the night and the day elimination rate was found to be statistically significant at the $p < 0.001$ level.[30] Much of this variation may be attributed to changes in the rate of glomerular filtration, which can result from dietary protein, salt and water balance, physical activity, and even emotional state.[29,36,38–39] Specifically, over the range of normal plasma chloride concentrations, the glomerular filtration rate will be altered by about 60%, increasing with higher salt intake.[36] Since CRE excretion parallels the glomerular filtration rate, any change in the latter would be expected to be reflected in the CRE excretion rate as well. Finally, the rate of tubular transport of CRE via secretion may vary during the day by as much as ± 35% (at 1 SD).[18] Clearly, each of the above internal processes could contribute to variability in daily CRE excretion.

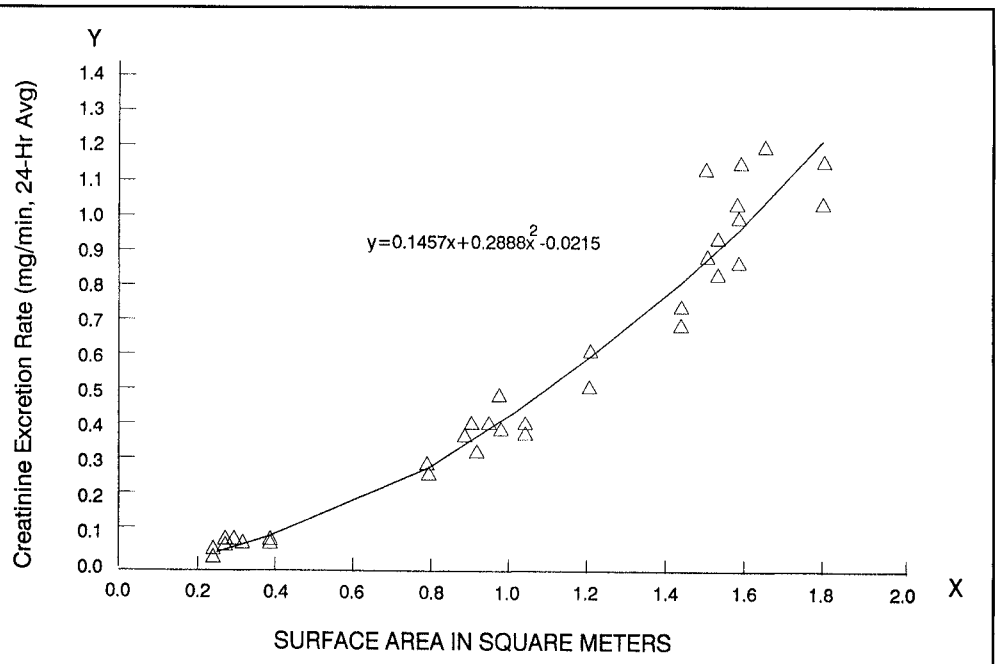

FIGURE 4. Empirical relationship between body surface area and creatinine excretion rate. Data adopted with permission from work of reference 18.

Body Mass

The 24-hour CRE elimination differs greatly among adult individuals. The expected range (at 95% confidence intervals) is between 0.5 to 3.0 grams per day—a six-fold range.[40] Because CRE is the product of the breakdown of creatine, found primarily in muscle mass, researchers have found a fairly good correlation between lean body mass (i.e., muscle) and CRE elimination.[41,42]

Since lean body mass is not precisely related to gross body weight, height, or to surface area, these parameters by themselves have not been shown to improve the correlation with CRE elimination.[41,43–45] However, in field practice, rough estimates of the expected CRE elimination can be made by using the above anthropometric parameters. A close estimate of human surface area can be calculated from weight and height by the formula[46]

$$\text{Body Surface Area} = 0.02350\ H^{0.42246} \times W^{0.51456} \qquad (2)$$

where surface area is in square meters, height is in centimeters (100 cm = 39.37 inch), and weight is in kilograms (1 Kg = 2.21 pounds). Urinary CRE has been empirically related to surface area by the relationship shown in Figure 4.[18]

Age and Sex

Among the other person-specific factors that have been used to assist in the estimation of expected CRE excretion, age and sex are probably the most well studied. These factors are important because of their general relationship to muscularity. In addition, the glomerular filtration rate also declines significantly with age.[47–48] Because of the general decline of muscularity and glomerular filtration rate with age, the average expected CRE elimination in a 65-year-old male is about 15 to 25% less than in a 20-year-old male.[40,49]

Females eliminate about 10% less CRE into their urine than men, given identical body weights, while they eliminate approximately 30 to 40% less CRE as a group than men, when the difference in weight is ignored.[50,51] Equations for calculating the daily production rate of CRE (R_{CRE}) from gross weight, age, and the sex of the individual, have been described previously.[51] A general equation developed for predicting 24-hour urinary CRE excretion, using 33 men, takes into account the lean body mass by requiring only gross weight and height.[52] The equation takes the form

$$R_{CRE}(gm) = 0.0143\ H_{cm} + 0.00975\ W_{kg} - 0.00734\ (age_{yr} - 20) - 1.391 \qquad (3)$$

and is estimated to have a predictive accuracy of ± 20% at the 95% confidence level. (If collecting short-term samples, one should expect wider deviations from this general predictor of CRE production.)

Health

Obviously, renal disease, especially when specifically related to glomerular filtration, will alter the CRE elimination

rate.[16,23,53] However, the presence of disease states in an individual and its influence on CRE elimination may not always be immediately apparent. Less obvious disease conditions that may affect the renal output of CRE include hypertensive-vascular disease, diabetes mellitus, hyperthyroid condition, and hepatic disease. Essential hypertension can result in a substantial increase in CRE secretion that is not reflective of the glomerular filtration rate.[54] Diabetes may be another condition that could lead to a transient elevation in the measured urinary CRE concentration, due to the positive interference from acetoacetate excretion.[55–56] The presence of acetoacetate in urine increases with diabetic ketoacidosis, which can be expected to be temporarily present in a portion of the diabetic population at all times.[57–59] Hyperthyroid conditions may either elevate or depress CRE clearance.[60] Finally, since the liver is one of the primary sources of creatine production, persons with hepatic disease may produce only half the CRE of normal persons.[14]

CRE excretion increases by 5–10% late in the second half of the menstrual cycle.[61] Pregnancy has been implicated to cause about a 50% increase in CRE production and excretion.[62,63] In addition, notable decreases in elimination have been reported in postmenstrual women.[43]

External Factors

Internal processes cannot by themselves account for the higher intra-day fluctuations that are seen. Additional external influences are involved. These include diet, diuresis, exercise, drugs, alcohol and tobacco, and incomplete voiding.

Diet

The primary influence of diet on urinary CRE elimination is a result of its meat content. This is not surprising, since meat contains creatine as well as traces of CRE. However, dietary creatine is not directly metabolized and eliminated as CRE, and the influence that different compounds have on the amount of CRE in urine has only recently become clear. It is now known that absorbed dietary creatine adds to the body's creatine pool and can accumulate over time.[64] Although the synthesis of endogenous creatine would be retarded, the quantity of CRE eliminated over time can gradually increase.[65]

In a specific example of the significance of meat consumption on CRE output, changes in the diet from a "high meat" diet containing about 0.9 g/day creatine to a "low meat" diet containing 0.1 g/day creatine contributed repeatedly to a 30 to 35% change in the urine CRE output in several individuals.[64] The change in excretion rate was apparently exponentially dependent on time, with half-lives of about two weeks. Other researchers have reported results that generally agree.[15,43,37,66–68] For the sake of comparison, if between 0.005 to 0.007 grams creatine per 1 gram of meat is assumed, a 16 ounce steak would contain 2.3 to 3.2 grams creatine.[64,69] Thus, by extrapolation, the potential for long-term elevations in CRE output, based only on diet, is considerable.

Conversely, dietary creatinine is readily absorbed from the intestine and is eliminated quickly from the blood by renal filtration.[17] Earlier experience suggested that the contribution of dietary CRE had insignificant short-term effects on the urinary concentration, since meat contains little CRE. This assumption was incorrect, since the analysis of CRE content was performed initially only on raw meat. (Also misunderstood were the differences between plant protein and meat protein.)[70] It is now known that cooking meat converts a substantial amount of its creatine to CRE.[68,69] Thus, a high-meat diet can quickly contribute substantial amounts of CRE to the blood that must then be eliminated.[45] One study determined that a single one-half pound of boiled beef elevated the CRE plasma concentration 52% within 1.5 to 3.5 hours after ingestion.[71,72] The return to the pre-meat urine output level can occur just as rapidly.[69] In one experiment involving the eating of a cooked meat meal, the CRE elimination dropped 48% in a subsequent five-hour period compared to the five-hour period just after eating the meal.[73]

As a final note on diet, although not usually related to workers, the effect of short-term fasting has been investigated, but the findings are contradictory.[74–75]

Diuresis

Normal daily urine volumes are about 1300 mL for women and 1450 mL for men.[76] Since the daily personal urine volume may commonly vary by up to 300% (600 to 2500 mL) or even more, this factor should be considered as a possible influence on CRE elimination. One author reported a statistically significant correlation between urine volume and CRE mass in successive 24-hour urine samples.[77] Many of the low creatinine excretion days corresponded to days when the average urine flow rate was low (less than 1 mL/min). While some recent work has demonstrated that urinary flow does affect the rate of CRE excretion,[85,85] the majority of the literature shows that when normal 24-hour urine volumes are compared to the total daily CRE excretion, little if any relationship is noted, as would be expected if the CRE excretion rate was truly independent of urine volume.[18,19,40]

Intra-day urine flow rates deviate substantially more than the longer inter-day urine output. Several researchers have found short-term declines in creatinine excretion that are associated with low urine flow.[77,78–83] The results of one of these studies are shown in Figure 5.[78] Work in hot environments will cause an increasing percentage of water loss through perspiration. When work breaks are infrequent and access to fluids is not readily available, urine flow rate may decrease essentially to zero.[89] Our own experience with industrial workers in normal temperature environments has been to occasionally see urine flow rates less than 0.2 mL/min. A potential problem arises if CRE is used to check the validity of a spot urine sample. Since a low urine flow may result in low CRE excretion, checking the CRE concentration in such a sample may not correctly indicate that it is a truly concentrated sample.

Exercise

The long-term effect of regular exercise is the increase of lean body mass. It may be expected that as a result of

regular exercise there will be an increase in the daily elimination of CRE of between 25 to 35% compared to a person of similar body weight and stature.[70]

Intense physical effort has been associated with affecting the short-term elimination of CRE in urine as well. Immediately after vigorous exercise, CRE elimination appears to fall. This initial decrease may be attributable to the normal decrement in renal blood flow and glomerular filtration rate while exercising.[92] The extent of fall equaled 33 to 36% in two experiments.[86–87] However, the elimination rate seems to quickly rebound and usually results in a net increase of up to 50% for the day.[19,34,86–88, 93]

Drugs, Alcohol and Tobacco

A number of drugs have been associated with decreased CRE elimination, among them cimetidine (duodenal ulcer treatment) and trimethoprim (antibacterial). Evidence suggests that these drugs compete for the organic cation transport mechanism that CRE uses and thus diminish its rate of secretion by the kidney.[90] Information of the effect of illicit drugs on CRE elimination was not found in the literature.

Chronic alcoholic beverage consumption was shown to have an inverse effect on serum CRE levels, and presumably CRE elimination. Although the difference was statistically significant, the change was only about 5% among the heaviest drinkers.[91] Later studies confirmed that the reduction in serum and urine CRE was directly linked to skeletal muscle myopathy and poor nutrition common to chronic alcoholics.[95]

Cigarette use has been associated with a small inverse effect on serum CRE, but the change was insignificant.[91]

Incomplete voiding

Some of the intra-day variability has been attributed to incomplete voiding of the urinary bladder. Problems with micturition, especially among older men affected by benign prostatic hypertrophy, are fairly common.[96] However, in normal men and women, an objective measurement of the significance of this contribution to the over-all variability has been reported to be much less than 1%.[94] Furthermore, because the CRE content of subsequent samples shows no compensation when the last result was high or low, one would think that the contribution of incomplete voiding is insignificant.[97] Rather, completely missed voids over a long period are of greater concern.[98] To perform a rough check of the completeness of urine samples for which the duration of collection is known, one might compare single-sample CRE concentrations to historical samples from an individual or use Equation 3 to calculate the expected CRE excretion rate.[99]

LIMITATIONS OF USING CRE WHEN ADJUSTING URINE RESULTS

The following section assumes that some knowledge about the process of eliminating a xenobiotic is known. In reality, renal excretion of most industrial compounds has not been studied in sufficient detail to identify the effect of hydration and other external factors on elimination. The mechanism of elimination can be studied provided that the compound may be administered to volunteer subjects.

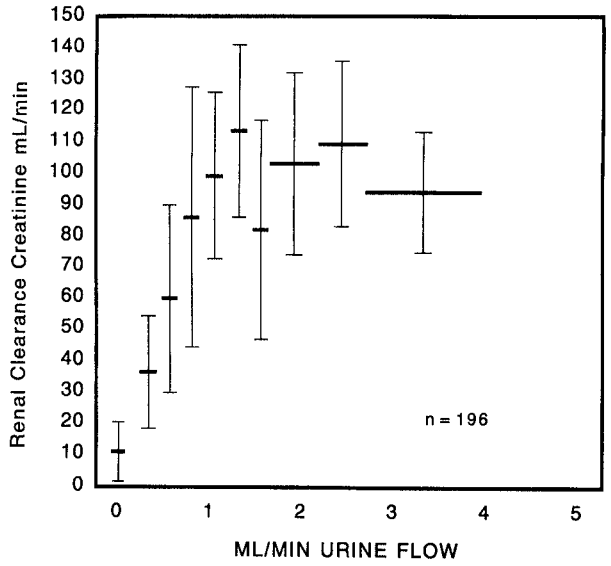

FIGURE 5. Effect of low urine flow rate on creatinine clearance in normal individuals. Mean clearance values, one standard deviation, and number of observations are presented at each flow rate. Reproduced with permission from work of reference 78.

One use of CRE has been to normalize untimed urine samples to the amount of CRE found in a given volume of urine. Assuming CRE is excreted at a constant rate, the amount of CRE in a spot sample could serve as a surrogate for timed samples. However, as has been shown by the foregoing reports, CRE is not excreted at a constant and predictable rate. Based on this limitation alone, the error on the expression of a urine substance concentration in short-term samples could be ± 200% or more, even with normal urine flow. Because of this error, a number of papers have appeared in the literature that have questioned using CRE to normalize untimed samples.[6,30,34,40,43,64,73,84,100–104]

For CRE to be appropriate in correcting the concentration of a xenobiotic for varying urine flow, the elimination of the xenobiotic must not be affected appreciably by urine flow. The differences in the mechanisms of renal clearance were presented previously and the effect on urine concentration exemplified in Figure 2. Several industrially important substances and their metabolites, including phenol, fluoride, and many volatile solvents are subject to renal reabsorption.[4,105–107] Under conditions of low urine flow (< 1 mL/min), elimination can dramatically decrease when the substances are eliminated by diffusion and are reabsorbed, whereas secreted substances and those not able to be reabsorbed may not be appreciably affected. Lead, for example, is reabsorbed to a greater extent during times of low urine flow, and adjusting the concentration in urine by the sample's specific gravity rather than it's CRE appears to minimize sample variability.[85,108–109]

CRE has been used to check the completeness of 24-hour urine collections. To be confident in this process, which may lead to a decision to eliminate certain samples from evaluation, one should have basic information on each individual and a history of their personal CRE elimination. Using CRE to check the completeness of short-term urine collections (as when determining the total amount of analyte excreted in a given time) would appear to have even less reliability, since the range of CRE excretion rate, especially between people, is so large.[52] This practice may only have utility in screening out extreme deviations from the expected. The use of empirical equations for predicting CRE excretion, such as Equation 3, may be more useful for screening sample results from each individual.

Low body hydration can occur during short-term monitoring and dramatically affect the excretion of CRE and some xenobiotics, which may be reason to exclude the results of such urine samples. But since CRE excretion may itself be significantly reduced during low urine flow, its concentration may be a poor indicator of the state of hydration and the adequacy of urine flow. Fortunately, urinary flow rate can be directly determined in timed urine collections.

An informal practice that should be questioned is the assumption that the "normal" urine CRE concentration for a "standard man" is about 1 g/L urine. This concentration has been used to convert uncorrected urine data to CRE corrected results and to convert spot samples to excretion rate results. The above quantity would appear inaccurate when we acknowledge the wide variability in urine flow rates. If CRE were actually excreted constantly over time (which is not correct), and were divided by the denominator of urine flow, which may have a range of 300% or more, one would expect a low degree of accuracy when converting raw urine data by the above "standard man" factor. One should not assume that the urine flow rate among persons working in the same location will be equivalent, since the amount of fluids consumed as well as the perspiration rate may be quite different. In reality, the concentration range of CRE in urine is expected to be over 1000%.[110]

ALTERNATE PROCEDURES TO CORRECT URINE SAMPLES

Body Size

Toxicokinetic studies show that the rate of intake (e.g., by respiration) of an exposure dose will depend on the size of the individual.[111,112] Adjustment of urine concentrations for total body mass should be particularly applicable when the substance is eliminated relatively constantly over time, and the excretion rate is dependent on the rate of intake and distribution, which are both influenced by body size. Attempts to factor in individual body size have generally been successful, sometimes halving the confidence width around the dose-excretion regression line.[113] Urinary CRE would be a generally good reflection of the muscle mass of the person exposed and would individualize by that perspective. However, muscle mass alone may not adequately correlate with total body mass and hence may not be strongly related to respiration and elimination. Other means of factoring in individual body size (e.g., expressing excretion in a given time per 1 kilogram of body weight) would be just as simple and possibly more meaningful when total mass (including adipose tissue) is involved with the uptake, storage, and distribution of the substance of interest.

Alternate means of expressing body mass could be used to help improve standardized expressions of substance dose for each person. Among the most popular measurements of body size are Body Surface Area (presented above in the text), and the Body Mass Index (BMI), which indicates adiposity and is calculated by dividing weight in kilograms by the square of the height in meters.[114] The latter index may be useful when the absorbed lipophilic chemical has an affinity for fat, which could affect the storage and elimination of such a compound from the body.

Expressions That Reflect Urine Flow Rate

Certainly variation in urine volume does not affect the rate of excretion of all substances equally. Inorganic mercury, for instance, has been found to be secreted almost constantly over time and is not reabsorbed.[85,108] Expressing untimed inorganic mercury concentrations by per-gram CRE would be an excellent use of the CRE adjustment if the excretion rate of CRE were stable over time. However, a time-integrated excretion rate was found to be a superior expression of internal dose.[85,115,116] CRE excretion is more variable than mercury excretion, since it is affected by several influences including urine flow. This makes the result of adjusting the urine mercury concentration by CRE less than optimal. To measure urine volume, which is necessary to know in order to relate the rate of excretion to a urine sample that is timed, the most convenient means is simply to weigh the sample on a metric scale, since the weight of urine is essentially 1 g/mL.

A means to correct this problem of variable urine flow and improve the adjustment of concentration has been proposed.[117] This involves adjusting the substance concentration to a standard urinary volume rate of 1 mL/min (1.4 L per day) and accounting for the influence of urine volume by the equation:

$$U_{(V=1)} = Ui \cdot Vi^{b} \quad (4)$$

where Ui = nonadjusted concentration, Vi = urinary flow rate (mL/min) and b = the value specific for each urinary substance calculated by the least squares method in the equation log Ui = a − b log Vi. Calculation of b is performed after several serial timed samples are collected from one or more persons. Testing of this approach on several metals and some endogenous organic compounds indicates that the affect of urine flow on concentration and the correlation of individual voids with the population mean is superior to the correlation provided by other common methods of expression, including the use of CRE.[11,85] This approach appears to have great promise for compounds where the rate of excretion varies with urine flow.

An identical approach to correcting the excretion rate of CRE for variable urine flow has been forwarded.[103] The empirical exponent value (which is equivalent to the slope of the log-log dependence of the creatinine excretion rate on urinary flow) that is used to adjust the urine flow rate is preliminary and has been reported by various authors to be 0.27–0.67.[118] At a urine flow rate of 0.5 mL/min. and 1.5 mL/min, this approach would decrease and increase the normal analyte concentration per gram CRE by about one-third, respectively. To obtain an independent value for the exponent, all samples must be collected and timed from multiple individuals for an extended period, and multiple linear regression with variables to account for inter-individual differences is necessary. However, this approach should improve the adjustment of urinary concentrations where CRE adjustments have already been shown to be appropriate or where it is preferred to express results by the CRE adjustment in following a historical precedent.

For substances in which the excretion rate is influenced by urine flow rate (i.e., concentration is less dependent on urine volume), adjusting the concentration to a standard specific gravity may help to minimize some of the variability associated with urine flow.[119] Although this approach may not be as effective as the above approaches, it can sometimes produce satisfactory results. Values for specific gravity can be expected to vary 10-fold (i.e., 1.003 to 1.030) and to correlate with urine flow rate.[110] However, it should be realized that specific gravity adjustments are also imprecise in reflecting urine flow rate and suffer from the same problems as CRE, being only convenient surrogates for timed samples.[6] Body size, protein in the diet, and sweating each may affect the rate of excretion of specific gravity-adjusted urine by a factor of about two.[119] Thus, at a given urine flow rate, the precision of the specific gravity from a group of persons may be expected to cover a range where the more concentrated sample will be up to eight times the least concentrated sample.

To calculate adjusted urine concentrations with specific gravity, one may use the following convenient formula:

$$\text{Corrected Concentration} = \text{Observed Concentration} \times \frac{24}{\text{Last 2 digits of sp. gr.}} \quad (5)$$

This formula assumes an average specific gravity of 1.024, while other values will also work if desired. One reported approach to urine expression first adjusts the raw urine concentration by specific gravity before expressing the final result as a rate of elimination.[120] Individual uptake and elimination could be further refined by relating the result to body size by some of the means suggested above.

DISCUSSION

When periodic collection of multiple timed urine samples from any individual is performed, a unique range of urine flow rates and creatinine excretion rates usually evolves. This range can be used to judge the normality of subsequent samples from an individual.[99]

When prior knowledge does not exist concerning the means by which a substance is excreted, and no attempt has been made to demonstrate whether adjusting the results can improve the expression of the data, it is first recommended that repeated samples be carefully collected from several individuals. Each of these sample results should be adjusted by the methods already reviewed and the final results either (1) compared to the overall group mean and the relative variance determined, or (2) compared to the measured dose (e.g., air concentration or calculated uptake). The method of expression providing the least variability about the mean or best correlation with exposure should then be selected.[6,109,113,115,121,122] A linear correlation between the analyte and CRE (or another selected denominator) should be observed using samples representing a wide range of concentrations if CRE is to be used to adjust all subsequent sample concentrations.

Attention to detail when collecting information on individual participants in urine-monitoring programs would reduce the level of uncertainty in the results, be more reflective of individual exposure, and result in fewer required samples, reduction in analytical cost, and better identification of specific workers at risk. Since the goal of biological monitoring is related to protecting workers' health, the emphasis should be on maximizing the accuracy of these results, not on simply providing convenience to the person collecting the samples when less than optimum data is the result.

One obstacle to adapting a new approach is in comparing the "new" results to historical results and to other results in the literature. However, all of the alternative methods described above simply "personalize" the excretion of a xenobiotic for an individual where the mass eliminated is adjusted by factors such as urine flow rate or body size. For the singular purpose of comparing results expressed by a new approach to historical results, a rough conversion of the "new" data back to the old means of expression could be performed using an adjustment factor based on the mean population value for that expression (e.g., analyte per gram CRE or per liter).

CONCLUSION

Because of the complexity of factors influencing the intake, distribution, and renal excretion of substances, interpreting urine results is difficult and not an exact science. Nevertheless, any means that take these influences into account and can be shown to improve the expression of personal internal dose should be promoted. Demonstration of the appropriateness of a means of expression should be performed in conjunction with a thorough review of the literature.

RECOMMENDATIONS

1. CRE may be used with some success to correct urine concentration results when the substance is eliminated primarily through renal filtration. CRE should not be applied to substances where passive elimination

and reabsorption, processes that are concentration dependent, are involved.

2. Before correcting urine concentrations with CRE, the user should be aware of the large potential variability of CRE excretion in short-term ($\leq$ 4 hour) voids and the special circumstances that may alter CRE excretion.

3. Workers should be well hydrated when collecting urine samples. If the urine flow rate falls below 1 mL/min., collections should ideally be repeated when the worker is better hydrated.

4. Alternative methods to using CRE correction as a means of expressing urinary elimination of a substance may correlate better with exposure, and should be explored.

5. Laboratory- and research-oriented field studies should consider the effect of hydration as well as other important physiological variables when collecting urine samples to ascertain the importance of these variables on excretion.

6. In order to relate urine results to internal dose, persons interpreting the results should be knowledgeable about the intake, storage, and distribution (i.e., pharmacokinetics) of the substance in the body. Samples should be collected in such a way that minimizes sample contamination and samples should be properly stored after collection.

ACKNOWLEDGMENTS

The many citations required for preparation of this manuscript were obtained due to the dedicated efforts of Mrs. Lucy Schoolfield and Mrs. Evelyn Palassis, NIOSH, Cincinnati. Technical and editorial review comments were received with great appreciation from Wayne Sanderson, Alexander W. Teass, Henryka U. Nagy, Michael J. Thun, Philip Edelman, Timothy J. Buckley, and Jennifer Boeniger.

REFERENCES

1. **Hill, D.L.:** Excretion of Diazotizable Metabolites in Man After Aniline Exposure. *A.M.A. Arch. Ind. Hyg. Occup. Med. 8*:347–349 (1953).
2. **Brenner, B.M.:** *The Kidney*, 3rd ed., ed. by B.M. Brenner, and F.C. Rector, (Eds.) Philadelphia: W.B. Saunders Co., (1986).
3. **Imbriani, M., S. Ghittori, G. Pezzagno, and E. Capodaglio:** Toluene and Styrene in Urine as Biological Exposure Indexes. *Ann. Am. Conf. Ind. Hyg. 12*:351–364 (1985).
4. **Ghittori, S., M. Imbriani, G. Pezzagno and E. Capadaglio:** The Urinary Concentration of Solvents as a Biological Indicator of Exposure: Proposal for the Biological Equivalent Exposure Limit for Nine Solvents. *Am. Ind. Hyg. Assoc. J. 48*:786–790 (1987).
5. **Sedivec, V., M. Marz and J. Flek:** Biological Monitoring of Persons Exposed to Methanol Vapors. *Int. Arch. Occup. Environ. Health 48*:257–271 (1981).
6. **Vesterberg, O., J. Sollenber, and K. Wrangskog:** Evaluation of Determinations Made in Urine Samples. Adjustments of Mandelic Acid Concentration Using Creatinine and Density. In: *Annals of the American Conference of Governmental Industrial Hygienists, International Symposium on Occupational Exposure Limits*, Cincinnati, OH: American Conference of Governmental Industrial Hygienists, 1985. pp. 301–304.
7. **Miettinen, J.K.:** Absorption and Elimination of Dietary Mercury (Hg^{2+}) and Methylmercury in Man. In *Mercury, Mercurials and Mercaptans*, ed. by M.W., Miller, T.W. Clarkson. Springfield, IL: Thomas, 1973.
8. **Araki, S.:** The Effects of Water Restriction and Water Loading on Urinary Excretion of Lead, Aminolevulinic Acid and Coproporphyrin. *Br. J. Ind. Med. 35*:312–317 (1978).
9. **Sonander, H., O. Stenqvist, and K. Nilsson:** Urinary N_2O as a Measure of Biological Exposure to Nitrous Oxide Anaesthetic Contamination. *Ann. Occup. Hyg. 27*:73–79 (1983).
10. **Rosier, J., H. Veulemans, R. Masschelein, M. Vanhoorne, and C. Van Peteghem:** Experimental Human Exposure to Carbon Disulfide. II. Urinary excretion of 2-Thiothiazolidine-4-carbosylic Acid (TTCA) During and After Exposure. *Int. Arch. Occup. Environ. Health 59*:243–250 (1987).
11. **Araki, S., O. Hiroshi, and M. Katsuyuki:** Adjustment of Urinary Concentration to Urinary Volume in Relation to Erythrocyt and Plasma Concentrations: An Evaluation of Urinary Heavy Metals and Organic Substances. *Arch. Environ. Health 41*:171–177 (1986).
12. **Foulkes, E.C.:** Mechanism of Renal Excretion of Environmental Agents. In *Handbook of Physiology*, ed. by D.H.K. Lee, Bethesda, MD: American Physiology Society, 1977.
13. **Piotrowski, J.:** *The Application of Metabolic and Excretion Kinetics to Problems of Industrial Toxicology*. Washington, D.C.: National Library of Medicine, 1971. pp 22–25.
14. **Cocchetto, D.M., C. Tschanz, and T.D. Bjornsson:** Decreased Rate of Creatinine Production in Patients with Hepatic Disease: Implications for Estimation of Creatinine Clearance. *Therap. Drug Monitor 5*:161–168 (1983).
15. **Crim, M.C., D.H. Calloway, and S. Margen:** Creatine Metabolism in Men: Urinary Creatine and Creatinine excretion with Creatine Feeding. *J. Nutr. 105*:428–438 (1975).
16. **Mitch, W.E., V.U. Collier, and M. Walser:** Creatinine Metabolism in Chronic Renal Failure. *Clin. Sci. 58*:327–335 (1980).
17. **Heymsfield, S.B., C. Arteaga, C. McManus, J. Smith and S. Moffitt:** Measurement of Muscle Mass in Humans: Validation of the 24-Hour Urinary Creatinine Method. *Am. J. Clin. Nutr. 37*:478–494 (1983).
18. **Dodge, W.F., L. Travis, and W. Daeschner:** Comparison of Endogenous Creatinine Clearance with Inulin Clearance. *Am. J. Dis. Child. 113*:683–692 (1967).
19. **Van Pilsum, J. and E. Seljeskog:** Long Term Endogenous Creatinine Clearance in Man. *Proc. Soc. Ex. Biol. Med. 97*:270–272 (1958).
20. **Renkin, E.M. and J.P. Gilmore:** Glomerular Filtration. In *Handbook of Physiology*, edited by J. Orloff, R.W. Berliner and S.R. Geiger. Washington D.C.: American Physiological Society, 1973. pp. 185–248.
21. **Tobias, G.J., R.F. McLaughlin, and J. Hopper:** Endogenous Creatinine Clearance: A Valuable Clinical Test of Glomerular Filtration and Prognostic Guide in Chronic Renal Disease. N. *Eng. J. Med. 266*:317–323 (1962).
22. **Blythe, W.B.:** The Endogenous Creatinine Clearance. Am. *J. Kidney Dis. 2*:321–323 (1983).

23. **Carrie, B.J., H.V. Golbetz, A.S. Maichaels and B.D. Myers:** Creatinine: An Inadequate Filtration Marker in Glomerular Diseases. *Am. J. Med. 69*:177–182 (1980)
24. **Bauer, L.A. and R.A. Blouin:** Influence of Age on Amikacin Pharmacokinetics in Patients Without Renal Disease. Comparison with Gentamicin and Tobramycin. *Eur. J. Clin. Pharmacol. 24*:639–642 (1983).
25. **Spencer, K.:** Analytical Reviews in Clinical Biochemistry: The Estimation of Creatinine. *Ann. Clin. Biochem. 23*:1–25 (1986).
26. **Bonsnes, R.W. and H.A. Taussky:** On the Colorimetric Determination of Creatinine by the Jaffe Reaction. *J. Biol. Chem. 158*:581 (1945).
27. **Technicon Method N-11a:** *Estimation of Creatinine Auto-Analyzer N Methodology*. Chauncy, NY: Technicon Instruments Corporation, 1963.
28. **Chasson, A., H. Grady, and M. Stanley:** Determination of Creatinine by Means of Automatic Chemical Analysis. *Am. J. Clin. Pathol. 35*:83–88 (1961).
29. **Richardson, J.A. and P.E. Philbin:** The One-Hour Creatinine Clearance Rate in Healthy Men. *J. Amer. Med. Assoc. 216*:987–990 (1971).
30. **Curtis, G. and M. Fogel:** Creatinine Excretion: Diurnal Variation and Variability of Whole and Part-Day Measures. *Psychosom. Med. 32*:337–350 (1970).
31. **Sawyer, W.T., B.R. Canaday, T.E. Poe, C.E. Webb, R.S. Porter, P. Gal, P.V. Joyner, J. Berry, S.W. Shearer, and C.V. Paoloni:** A Multicenter Evaluation of Variables Affecting Predictability of Creatinine Clearance. *Am. J. Clin. Pathol. 12*:832–838 (1982).
32. **Narayanan, S. and H.D. Appleton:** Creatinine: A Review. *Clin. Chem. 26*:1119–1126 (1980).
33. **Gowans, E.M. and C.G. Fraser:** Biological Variation of Serum and Urine Creatinine and Creatinine Clearance: Ramifications for Interpretation of Results and Patient Care. *Ann. Clin. Biochem 25*:259–263 (1988).
34. **Greenblatt, D.J., B.J. Ransil, J.S. Harmatz, T.W. Smith, D.W. Duhme, and J. Koch-Weser:** Variability of 24-Hour Urinary Cratinine Excretion by Normal Subjects. *J. Clin. Pharmacol. 16*:321–328 (1976).
35. **Sirota, J.H., D.S. Baldwin, and H. Villarreal:** Diurnal Variations of Renal Function in Man. *J. Clin. Invest. 29*:187–192 (1950).
36. **Wesson, L.G. and D.P. Lauler:** Diurnal Cycle of Glomerular Filtration Rate and Sodium and Chloride Excretion During Responses to Altered Salt and Water Balance in Man. *J. Clin. Invest. 40*: 1967–1977 (1961).
37. **Banda, P.W., M.S. Tuttle, A.E. Sherry and M.S. Blois:** Total Creatinine Content of the First Morning Urine is Independent of Dietary Changes. *Clin. Chem.* 26:6851–6852 (1980).
38. **Bosch, J.P., A. Saccaggi, A. Lauer, A., C. Ronco, M. Belledonne, and S. Glabman:** Renal Functional Reserve in Humans. Effect of Protein Intake on Glomerular Filtration Rate. *Am. J. Med.75*:943–950 (1983).
39. **Brod, J:** Regulation of Renal Function. *Acta Med. Acad. Sci. Hung. 4*:369–395 (1953).
40. **Alessio, L., A. Berlin, A. Dell'Orto, F. Toffoletto, and I. Ghezzi:** Reliability of Urinary Creatinine as a Parameter Used to Adjust Values of Urinary Biological Indicators. *Int. Arch. Occup. Environ. Health 55*:99–106 (1985).
41. **Edwards, K.D.G. and H.M. Whyte:** Creatinine excretion and Body Composition. *Clin. Sci. 18*:361–366 (1959).
42. **Fuller, L. and A.J. Rich:** An Index of Lean Body Mass from 24h Urinary Creatinine Excretion. *Proc. Nutr. Soc. 41*:A104 (1982).
43. **Hyde, E.:** Creatine Feeding and Creatine-Creatinine Excretion in Males and Females on Different Age Groups. *J. Biol. Chem. 143*:301–310 (1942).
44. **Rosano, T.G. and H.H. Brown:** Analytical and Biological Variability of Serum Creatinine and Creatinine Clearance: Implications for Clinical Interpretation. *Clin. Chem. 28*:2330–2331 (1982).
45. **Bleiler, R. and H. Schedl:** Creatinine excretion: Variability and Relationships to Diet and Body Size. *J. Lab. Clin. Med. 59*:945–955 (1962).
46. **Gehan, E.A. and S.L. George:** Estimation of Human Body Surface Area from Height and Weight. *Cancer Chemother. Rep. Part I 54*:225–235 (1970).
47. **Gault, M.H. and D.W. CockCroft:** Creatinine Clearance and Age. *Lancet 2*:612 (1975).
48. **Rowe, J.W., R. Andres, J.D. Tobin, A.H. Norris, and N.W. Shock:** Age-Adjusted Standard for Creatinine Clearance. *Ann. Intern. Med. 84*:567–569 (1976).
49. **Driver, A.G. and M.T. McAlevy:** Creatinine Height Index as a Function of Age. *Am. J. Clin. Nutr. 33*:2057 (1980).
50. **Simpson, F.O., E.R. Nye, P. Bolli, H.J. Waal-Manning, A.W. Goulding, E.L. Phelan, F.A. de Hamel, R.D.H. Stewart, G.F.S. Spears, G.M. Leek, and A.C. Stewart:** The Milton Survey: Part 1, General Methods, Height, Weight and 24-Hour Excretion of Sodium, Potassium, Calcium, Magnesium and Creatinine. *New Eng. J. Med. 87*:379–382 (1978).
51. **Bjornsson, T.D.:** Use of serum Creatinine Concentrations to Determine Renal Function. *Clin. Pharmacokinet. 4*:200–222 (1979).
52. **Turner, W.J. and S. Cohn:** Total Body Potassium and 24-Hour Creatinine Excretion in Healthy Males. *Clin. Pharm. Therap. 18*:405–412 (1975).
53. **Singh, A., K.S. Chugh, and B.K. Sharma:** Three Hour Endogenous Creatinine Clearance as a Test of Glomerular Filtration in Normal Subjects and Patients with Chronic Renal Failure. *Ind. J. Med. Sci. 33*:61–64 (1979).
54. **Berlyne, G.M., H. Varley, S. Nilwarangkur, and M. Hoerni:** Endogenous-Creatinine Clearance and Glomerular-Filtration Rate. *Lancet 2:*847–848 (1964).
55. **Glick, M.R., W.R. Moorehead, T.O. Oei, and G.R. Moore:** Acetoacetate and "Ketone" Interference in Kinetic and Continuous Flow Methods for Creatinine. *Clin. Chem. 26:* 1626–1627 (1980).
56. **Blank, D.W. and A.A. Nanji:** Ketone Interference in Estimation of Urinary Creatinine; Effect on Creatinine Clearance in Diabetic Ketocidosis. *Clin. Biochem. 15*:279–280 (1982).
57. **Boulton, A.J., G. Knight, J. Drury, and J.D. Ward:** Diabetic Ketoacidosis Associated with Outpatient Treatment Using Continuous Subcutaneous Insulin Infusion. *Postgrad. Med. J. 59*:438–439 (1983).
58. **Malone, J.T., J.M. Hellrung, E.W. Malphus, A.L. Rosenbloom, A. Grgic, and F.T. Weber:** Thomas Weber: Good Diabetic Control—A Study in Mass Delusion. *J. Pediatr. 88*:943–947 (1976).
59. **Wahren, J., L. Hagenfeldt:** Free Fatty Acid and Ketone Body Metabolism During Exercise in Diabetes. *Acta. Paediatr. Scand. (suppl.)283*:39–44 (1980).
60. **Mintz, D.H., D.E. Hellman, and J.J. Canary:** Effect of Altered Thyroid Function on Calcium and Phosphorus

Circadium Rhythms. *J. Clin. Endocrinol. Metab. 28*:399–411 (1968).
61. **Smith, O.W.:** Creatinine Excretion in Women: Data Collected in the Course of Urinalysis for Female Sex Hormones. *J. Clin. Endocrinol. 2*:1–12 (1942).
62. **Brod, J. and J.H. Sirota:** The Renal Clearance of Endogenous Creatinine in Man. *J. Clin. Invest. 27*:645 (1948).
63. **Sims, E.A.H. and Krantz, K.E.:** Serial Studies of Renal Function During Pregnacy and Puerperium in Normal Women. *J. Clin. Invest. 37*:1764 (1958).
64. **Lykken, G.I., R.A. Jacob, J.M. Muno, and H.H. Sandstead:** A Mathematical Model of Creatine Metabolism in Normal Males—Comparison between Theory and Experiment. *Am. J. Clin. Nutr.* 33:2674–2685 (1980).
65. **Hoberman, H.D., E.A.H. Sims, and J.H. Peters:** Creatine and Metabolism. *Biol. Chem. 172*:45–58 (1948).
66. **Rose, W.C. and F.W. Dimmitt:** Experimental Studies on Creatine and Creatinine. VII. The Fate of Creatine and Creatinine when Administered to *Man. J. Biol. Chem. 26*:345–353 (1916).
67. **Sangoi, J.K. and B.S. Kulkarni:** Creatinine Clearance Studied in Normal Indian Subjects. *J. Postgrad. Med. (Bombay) 18*:189–198 (1972).
68. **Greene, E., B.J. Hoogwerf, and D. Laine:** Diet Composition Affects Urine Creatinine Excretion in Healthy Subjects. *Clin. Res. 30*:A779 (1982).
69. **Camara, A.A., K.D. Arn, A. Reimer, and L.H. Newburgh:** The Twenty-four Hourly Endogenous Creatinine Clearance as a Clinical Measure of the Functional State of the Kidneys. *J. Lab. Clin. Med. 37*:743–763 (1951).
70. **Jackson, S:** Creatinine in Urine as an Index of Urinary Excretion Rate. *Health Physics 12*:843–850 (1966).
71. **Jacobsen, F., C. Christensen, C. Mogensen, F. Andreason, and N.S.C. Heilskov:** Pronounced Increase in Serum Creatinine Concentration After Eating Cooked Meat. *Br. Med. J. 21*:1049–1050 (1979).
72. **Mayersohn, M., K. Conrad, and R. Achari:** The Influence of Cooked Meat Meal on Creatinine Plasma Concentrations and Creatinine Clearance. *Br. J. Clin. Pharmac. 15*:227–230 (1983).
73. **Sherman, R.A.:** Creatinine in the Crock Pot. *Arch. Int. Med. 141*:543 (1981).
74. **Pasternack, A. and B. Kuhlback:** Diurnal Variations of Serum and Urine Creatine and Creatinine. *Scand. J. Clin. Lab. Invest. 27*:1–7 (1971).
75. **Tocci, P.M., J. Phillips, and R. Sager:** The Effect of Diet Upon the Excretion of Parahydroxy Phenylacetic Acid and Creatinine in Man. *Clin. Chim. Acta 40*:449–453 (1972).
76. **Knuiman, J.T., A.J. Hautvast, L. Van Der Heyden, J. Geboers, J.V. Joossens, H. Tornquist, B. Isaksson, P. Pietinen, U. Tuomilehto, L. Poulsen, A. Flynn, C. Shortt, H. Böing, B. Yomtov, E. Magalhaes, F. Angelico, C. Stefanutti, C. Fazio, R. Cantini, G. Ricci, A. Trichopoulou, and J. Katapoti:** A Multi-Center Study on Completeness of Urine Collection in 11 European Centres. *Human Nutr. Clin. Nutr. 40C*:229–237 (1986).
77. **Chattaway, F.W., R.P. Hullin, and F.C. Odds:** The Variability of Creatinine Excretion in Normal Subjects, Mental Patients and Pregnant Women. *Clin. Chim. Acta 26*:567–576 (1969).
78. **Vree, T.B., Y.A. Hekster, C.M. Hafkenscheid, R. van Dalen, and W. T. Friesen:** The Influence of Urine Flow on Renal Clearance of Creatinine in Patients with Normal and Impaired Kidney Function. *Drug Intell. Clin. Pharm. 15*:194–198 (1981).
79. **Winter, K.A., G. Wisfeld and T. Knauth:** Zur Kreatinin und Paraaminohippursure-Ausscheidung bei Weshselnder Nierentatigkeit [More on Creatinine and Para-aminohippuric Acid Excretion in Various Kidney Functions.] *Ztschr. ges. inn. Med. 9*:404–408 (1954). [German]
80. **Heidlemann, G., E. Koch, and M. Haake:** Die Abhangigkeit der Ausscheidung des Endogenen Creatinins und des Phenolrots von der Diurese [The Excretion of Endogenous Creatine and Phenol caused by Diuresis]. *Verhandl. deutsch. Gesellsch. inn. Med. 62*:621–23 (1956). [German]
81. **Gayer, J. and J. Kramer:** Die Tubulare Sekretion von Endogenem Kreatinin [The Tubular Secretion of Endogenous Creatinine]. *Klin. Wchnschr. 35*:626–31 (1957). [German]
82. **Van Dalen, R., T.B. Vree, J.C.M. Hafkenscheid, and J.S.F. Gimbrere:** Determination of Plasma and Renal Clearance of Cefuroxime and its Pharmacokinetics in Renal Insufficiency. *J. Antimicrob. Chemother. 5*:281–292 (1979).
83. **Mayersohn, M., K.A. Conrad, and R. Achari:** Creatinine Clearance and Urine Flow. Drug Intel. *Clin. Pharm. 17*:131 (1983).
84. **Bailey, R. and H. de Wardener:** Creatinine Excretion. *Lancet i (Jan. 17)*:145 (1970).
85. **Araki, S., F. Sata and K. Murata:** Adjustment for Urinary Flow Rate: An Improved Approach to Biological Monitoring. *Int. Arch. Occup. Environ. Health 62*:471–477 (1990).
86. **Radha, E. and S.P. Bessman:** Effect of Exercise on Protein Degradation: 3-Methylhistidine and Creatinine Excretion. *Biochem. Med. 29*:96–100 (1983).
87. **Decombaz, J., P. Reinhardt, K. Anantharaman, G. von Glutz, and J.R. Poortmans:** Biochemical Changes in a 100 Km. Run: Free Amino Acids, Urea, and Creatinine. *Eur. J. Appl. Physiol. 41*:61–72 (1979).
88. **Zorbas, Y., V. Petrovskty, and Y. Koichi:** Creatinine Excretion Under Physical Exercise and Hypokinesia. *Ergonomics 25*:461 (1982).
89. **Parmeggiani, L. and C. Sassi:** Lavoro Pesante in Miniera in condizioni Caldo-umide. Parte Prima: Adattamento Funzionale, Termoregolazione ed Equilibrio Idrico [Heavy Work in Mine Stopes with Hot, Humid Conditions. First Report: Functional adaptation, Thermoregulation, and Water Balance]. *Med. Lav. 46*:295–324 (1955) [Italian]
90. **Preuss, H.G.:** Organic Cationic Drugs and Renal Creatinine Secretion. *Renal Physiol. 6*:103 (1983).
91. **Savdie, E., G. Grosslight, and M. Adena:** Relation of Alcohol and Cigarette Consumption to Blood Pressure and Serum Creatinine Levels. *J. Chron. Dis. 37*:617–634 (1984).
92. **Nichols, J.M., A.T. Miller, and E.P. Hiatt:** Influence of Muscular Excercise on Uric Acid Excretion in Man. *Appl. Physiol. 3*:501–507 (1951).
93. **Srivastava, S.S., K.V. Mani, and C.M. Soni:** Effect of Muscular Exercise on Urinary Excretion of Creatine and Creatinine. *Ind. J. Med. Res. 55*:953–960 (1957).
94. **Scott, P.J. and J. Hurley:** Demonstration of Individual Variation in Constancy of 24-Hour Urinary Creatinine Excretion. *Clin. Chim. Acta 21*:411–414 (1968).
95. **Duane, P. and T. Peters:** Nutritional Status in Alcoholics with and Without Chronic Skeletal Muscle Myopathy. *Alcohol & Alcoholism 23*:271–277 (1988).

96. **Herbison, A.E., M.R. Fraundorfer, and J.K. Walton:** Association Between Symptomatology and Uroflowmetry in Benign Prostatic Hypertrophy. *Br. J. Urol. 62:*427–430 (1988).
97. **Schottstaedt, W.W., W.J. Grace, and H.G. Wolff:** Life Situations, Behaviour, Attitudes, Emotions, and Renal Excretion of Fluid and Electrolytes—V. Variations in Excretion of Endogenous Creatinine. *J. Psycosomat. Res. 1*:292–298 (1956).
98. **Wheeler, L. and L. Sheiner:** Clinical Estimation of Creatinine Clearance. *Am. J. Clin. Pathol. 72*:27–31 (1979).
99. **Boeniger, M.:** Industrial Hygiene Report on the General Electric Plant, Albuquerque, New Mexico. (IWSB Report IHS 143.21), Cincinnati, OH: National Institute for Occupational Safety and Health, 1990.
100. **Paterson, N.:** Relative Constancy of 24-Hour Urine Volume and 24-Hour Creatinine Output. *Clin. Chim. Acta 18*:57–58 (1967).
101. **Edwards, O., R. Bayliss, and S. Millen:** Urinary Creatinine Excretion as an Index of the Completness of 24-Hour Collections. *Lancet ii (Nov. 29)*:1165–1166 (1969).
102. **Vestergaard, P. and R. Leverett:** Constancy of Urinary Creatinine Excretion. *J. Lab. Clin. Med. 2*:211–218 (1958).
103. **Greenberg, G.N. and R.J. Levine:** Urinary Creatinine Excretion is Not Stable: A New Method for Assessing Urinary Toxic Substance Concentrations. *J. Occup. Med. 31*:832–838 (1989).
104. **Ram, M.:** Variability in Urinary Creatinine. *Lancet ii (Sept. 26)*:674 (1970).
105. **Ekstrand, J., C.J. Spak, and M. Ehrnebo:** Renal Clearance of Fluoride in a Steady State Condition in Man: Influence of Urinary Flow and pH Changes by Diet. *Acta Pharmacol. Toxicol. 50*:321–325 (1982).
106. **Hunter, C.G.:** Solvents with Reference to Studies on the Pharmacodynamics of Benzene. *Proc. Royal Soc. Med. 61*:913–915 (1968).
107. **Carlson, C., W.D. Armstrong, and L. Singer:** Distribution and Excretion of Radiofluoride in the Human. *Proc. Soc. Exp. Biol. Med. 104*:235–239 (1960).
108. **Araki, S., H. Aono, K. Yokoyama, and K. Murata:** Filterable Plasma Concentration, Glomerular Filtration, Tubular Balance, and Renal Clearance of Heavy Metals and Organic Substances in Metal Workers. *Arch. Environ. Health 41*:216–221 (1986).
109. **Ellis, R.W.:** Urinary Screening Tests to Detect Excessive Lead Absorption. *Br. J. Ind. Med.* 23:263–281 (1966).
110. **Wallach, J.:** *Interpretation of Diagnostic Tests.* Boston: Little, Brown & Co., 1986.
111. **Zielhuis, R.L.:** Approaches in the Development of Biological Monitoring Methods: Laboratory and Field Studies. In *Biological Monitoring and Surveillance of Workers Exposed to Chemical*, ed. by A. Aitio et al., New York: Hemisphere Publishing, 1984.
112. **Doull, J.:** Factors Influencing Toxicology. In *Casarett and Doull's Toxicology, the Basic Science of Poisons*, ed. by J. Doull, C.D. Klaassen, and M.O. Amdur. New York: Macmillan Publishing, 1980. pp. 70–83.
113. **Sedivec, V. and J. Flek:** Exposure Test for Xylenes. *Int. Arch. Occup. Environ. Health 37*:219–232 (1976).
114. **Bray, G.A.:** Overweight is Risking Fate: Definitions, Classification, Prevalence, and Risks. Monograph 499 on Human Obesity. Ann. NY Acad. Sci., 1987. pp. 14–28.
115. **Aitio, A., S. Valkonen, H. Kivisto, and E. Yrjanheikki:** Effect of Occupational Mercury Exposure on Plasma Lysosomal Hydrolases. *Intern. Arch. Occup. Environ. Health 53*:139–147 (1983).
116. **Yokoyama, K., S. Araki, and R. Yamamoto:** Renal Handling of "Filterable" Plasma Metals and Organic Substances in Man. *J. Appl. Tox. 5*:94–96 (1985).
117. **Araki, S.:** Effects of Urinary Volume on Urinary Concentrations of Lead, Aminolaevulinic Acid, Coproporphyrin, Creatinine, and Total Solutes. *Br. J. Ind. Med. 37*:50–54 (1980).
118. **Verplanke, A.J., R.F. Herber, and J.P. Straub:** Creatinine Concentration and Urine Flow: Letter to the Editor. *J. Occup. Med. 32*:763–766 (1990).
119. **Elkins, H.B., L.D. Pagnotto, and H.L. Smith:** Concentration Adjustments in Urinalysis. *Am. Ind. Hyg. Assoc. J. 35*:559–565 (1974).
120. **Wilczok, T. and G. Bieniek:** Urinary Hippuric Acid Concentration After Occupational Exposure to Toluene. *Br. J. Ind. Med. 35*:330–334 (1978).
121. **Ogata, M., K. Tomokuni, and Y. Takatsuka:** Urinary Excretion of Hippuric Acid and m- or p-Methylhippuric Acid in the Urine of Persons Exposed to Vapors of Toluene and m- or p-Xylene as a Test of Exposure. *Br. J. Ind. Med. 27:*43–50 (1970).
122. **Ikeda, M. and H. Ohtsuji:** Hippuric Acid, Phenol, and Trichloroacetic Acid Levels in the Urine of Japanese Subjects With No Known Exposure to Organic Solvents. *Br. J. Ind. Med. 26*:162–164 (1969).