

Review Article

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Expressing the quantity of urinary analytes: a discussion of some issues arising from the monitoring of the menstrual cycle

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Abstract: Practical domestic monitoring of the menstrual cycle requires measurements of urinary metabolites of reproductive hormones: oestrone glucuronide (E1G) and pregnanediol glucuronide (PdG). Data reported in the literature are expressed as (i) concentration, without or with either creatinine- or specific gravity correction, or (ii) excretion rates. This variation in such a fundamental issue prompts consideration of the relationships between the four measures. Because the menstrual cycle kinetics of E1G and PdG are complex, we consider measurements of urinary creatinine, urea, galactose, xylose and inulin which tend to be more stable. We show that uncorrected concentration measurements of these urinary analytes can be positively correlated, negatively correlated or uncorrelated with the serum concentration. Based on measurements of urinary creatinine concentrations, urinary specific gravity and creatinine excretion rates, we conclude that urinary analyte concentration are likely to be more reliable when creatinine-corrected rather than corrected using specific gravity, but that both are less reliable than measurements of the excretion rate. This has implications for the quantitation of any urinary analyte, but especially for the monitoring of the menstrual cycle in which changes in E1G and PdG from one day to the next can be physiologically significant for a woman monitoring her fertility.

Keywords: Concentration; Creatinine; Excretion rate; Menstrual cycle; Specific gravity; Urine

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1 Introduction

It is widely accepted that the menstrual cycle can be monitored by following the serum concentrations of oestradiol (E2) and progesterone (P) [1]. However, there are several objections to this, of which two are of particular significance. First, the daily blood sampling required is unacceptable to women, so it is more usual to monitor the urinary metabolites of E2 and P instead [2]. The most common metabolites to be used in this way are, respectively, oestrone 3-glucuronide (E1G) and pregnanediol glucuronide (PdG) [3-8]. Second, in monitoring the menstrual cycle what is actually relevant is the rate at which E2 and P are produced because that provides an insight into the dynamics of the process. For example, the rate of E2 secretion increases with the diameter of the growing follicle and secretion ceases on ovulation, or shortly thereafter, and increases again as the corpus luteum develops. In this context the serum E2 concentration merely reflects the balance between E2 secretion and its speciation (into oestrone (E1) and oestriol (E3) and their various glucuronides and sulphonates) and excretion. The rate of excretion of E1G, for example, is more reliably related to the rate of E2 secretion than it is to the E2 serum concentration [9].

While it is agreed that E1G and PdG are practical urinary analytes to monitor for this purpose, there is some debate about what should actually be measured. In practice, there are at least four common alternatives: (i) the analyte concentration [6, 7], (ii) concentration corrected by normalising to the creatinine concentration [10, 11], (iii) concentration corrected by normalising to the specific gravity [10, 11], or (iv) the analyte excretion rate [3, 4, 8]. On occasion, urinary analyte concentrations have been corrected by normalising to osmolality [12, 13], but this is not common among reports of E1G and PdG levels. For completeness, we note that mathematical approaches to correction have also been proposed [14-16] and many have tried to avoid the problem by using concentration ratios of urinary analytes including E1G and PdG [17-24].

These measures have different requirements and tend to be used for slightly different purposes. Concentration is used to obtain an approximate indication of the events in the menstrual cycle. It is the basis of several widely used commercial tests that rely on measurements of reproductive hormones. It is claimed that women can use these as an aid in achieving or delaying pregnancy (we have recently reanalysed data on which some of these claims are based[25]). The creatinine-normalised concentration is used for more detailed comparisons of cycles and depends on laboratory-based measurements. The specific gravity-normalised concentration has often been used for epidemiological research and it also requires laboratory instruments. Measurements based on excretion rates can provide a woman with detailed information about her own cycle and she can carry them out in her own home [3-5, 26-28].

Given their significance to so many women and their partners, it is important to consider the limitations of these commonly used measures of E1G and PdG. However, even in a 'normal' menstrual cycle these analytes have very complex kinetics [8] because the events in the ovaries from which E2 and P come result from interactions with the pituitary gland involving other hormones (including luteinising and follicle-stimulating hormones). As there is nothing unique about the excretion of E1G and PdG we choose to clarify some of the issues associated with the various measures by considering analytes that have fewer of the complexities associated with the menstrual cycle. Specifically, we consider measures of urinary creatinine, inulin, xylose, galactose and urea for which data are available in the literature. Of these, creatinine is usually assumed to be excreted at a constant rate, inulin is considered metabolically inert and xylose is largely unmetabolised. We begin by defining the measures and examining the connections between them and considering the transfer of solutes from the blood to the urine. Then we use simple examples to clarify some of the issues associated with the use of these different measurements. While the approach we take arises from our work involving the metabolites of reproductive hormones, the treatment we outline (and our conclusions) are completely general.

2 Theoretical background

2.1 Concentration

The urinary concentration of analyte A (u_A) is just the quantity of that analyte (q_A) per unit volume of urine (V)

$$u_A = \frac{q_A}{V} \quad (1)$$

(the corresponding serum concentration of A (s_A) is defined similarly). Each of us knows from personal experience that the volume of urine (V) can fluctuate considerably from void to void. Our own data indicate that the distribution of the volume of overnight urine samples tends to be positively skewed and ranges from about 50 mL to approximately 1 L [29] and other data have a similar distribution, although V can have an even greater range (Brown, Cooke and Blackwell, manuscript in preparation) and if, as is often the case, circumstance dictates that daytime rather than overnight samples are sometimes collected then the variation can be increased further. Based on our overnight data, two samples containing exactly the same quantity of an analyte can differ in concentration by about 20-fold just because of fluctuations in V [29]. The inevitable question is whether such a difference in u_A has any physiological significance. In spite of these uncertainties, reports of the use of concentration measurements to monitor reproductive hormones in urine are increasingly common and in some it is claimed that specific threshold values are associated with particular events in the cycle [2, 6, 7, 30-46]. In none of these cases were the data of a single cycle used by a woman to monitor her own fertility. Our own data indicate that that physiological interpretation of a sequence of daily concentration-based measurements can be very challenging [9]. Instead, averaged cycles, usually based on data obtained from many women, were employed which prompts important questions some of which we will consider elsewhere (Cook, Blackwell and Brown, manuscript in preparation).

2.2 Excretion rate

The excretion rate of analyte A (J_A) is the quantity of A excreted per unit time. This is usually estimated as the amount accumulated in the volume of urine accumulated in the time since the previous void (Δt)

$$J_A = \frac{q_A}{\Delta t} \quad (2)$$

It will be appreciated that J_A can also be viewed as a function of both u_A and the urine production rate ($J_V = V/\Delta t$)

$$J_A = u_A J_V \quad (3)$$

from which it follows that the concentration of an analyte (1) is also

$$u_A = \frac{J_A}{J_V} \quad (4)$$

We have shown that the distribution of J_V of overnight urine samples tends to be positively skewed and ranges from about 10 mL h⁻¹ to approximately 200 mL h⁻¹ [29]. Based on these data, for two samples having exactly the same analyte concentration J_A can vary about 20-fold just because of fluctuations in J_V [29]. Despite this, it is not uncommon to find reports in which J_V is assumed to be constant [47], uniformly distributed [48] or normally distributed [49]. For example, Karpas *et al.* [47] simply estimated uranium excretion rates by multiplying each urinary concentration by 1.4 L d⁻¹ (3), which they assumed was the ‘population’ average J_V .

2.3 Creatinine-corrected concentration

The urinary concentration of an analyte is often corrected using the concentration of creatinine (u_{Cr}) in the same urine sample. The most frequent approach is to divide u_A by u_{Cr} , although other creatinine corrections have been used for urinary analytes other than reproductive hormones [50-52]. It will be appreciated from (1-4) that the simplest form of the creatinine-corrected concentration can be viewed interchangeably as a relative concentration, a relative quantity or a relative excretion rate

$$\frac{u_A}{u_{Cr}} = \frac{q_A}{q_{Cr}} = \frac{J_A}{J_{Cr}} \quad (5)$$

The concept of creatinine-correction is based on the work of Otto Folin [53-55] who analysed the 24 hour urines of institutionalised individuals kept on the same strict diet and fluid intake for several days. He observed “...the remarkable fact that *the absolute quantity of kreatinin eliminated in the urine on a meat-free diet is a constant quantity different for different individuals, but wholly independent of quantitative changes in the total amount of nitrogen eliminated*. This appears ... to be another fixed principle in the chemistry of metabolism” [55: 84, his italics]. The widely held view that the rate of creatinine output ($J_{Cr} = u_{Cr} J_V$) is constant is ultimately based on this, although Ohira *et al.* [56] show how unreliable this generalisation can be. Nevertheless, it follows from this assumption that variations in u_{Cr} are inversely related to J_V and that

$$J_A = \frac{u_A}{u_{Cr}} J_{Cr} \propto \frac{u_A}{u_{Cr}} \quad (6)$$

but the basis of the widespread use of this form of creatinine correction neglects Folin’s observation that J_{Cr} is “.. a constant quantity *different for different individuals*” on a meat-free diet [55 p. 84, our italics]. Not only does the J_{Cr} differ between subjects, but it can also vary considerably for a single subject. For example, J_{Cr} depends on diet [57], exercise, age, muscle mass and BMI [58, 59], and there is some evidence that it varies diurnally [60, 61] and during the course of the menstrual cycle [62-71].

Creatinine correction is very widely used for various urinary analytes and it is often suggested that urine samples that are too dilute ($u_{Cr} < 0.5$ g/L) or too concentrated ($u_{Cr} > 3$ g/L) should be discarded [72], although other limits are sometimes used [56, 73, 74]. Such samples are not uncommon, for example, Gaines *et al.* [75] report that 43 or 29 of 417 samples (a total of 17%) were too dilute ($u_{Cr} < 0.5$ g/L) or too concentrated ($u_{Cr} > 3$ g/L), respectively. For data we have previously reported [76], 13% of samples provided by 12 women over 26 menstrual cycles would have been excluded from further consideration on this basis. To put this in context, a woman monitoring her menstrual cycle might expect to exclude one of her daily samples each week just because u_{Cr} is not within the range. As women rely on such measurements to provide information on which they base potentially significant decisions, this is not desirable. However, the main practical problem with this mode of correction in monitoring the menstrual cycle is that it requires that creatinine is assayed in addition to E1G and PdG, a combination that has not yet been established for home use. Nevertheless, it is likely that this approach will be tried and so we include some consideration of creatinine correction below (see section 5.5, particularly).

2.4 Specific gravity-corrected concentration

An alternative means of correcting u_A is usually described as being based on the specific gravity (SG) of the urine. In practice, SG is usually estimated using a refractometer rather than a hydrometer [10] because there is a good correlation between the SG and refractive index of urine [77]. This correction has been implemented in various ways, but in each case the concentration is normalised to a function of the specific gravity ($f(SG)$) of the urine. One common form of the specific gravity-corrected concentration is

$$u_A f(SG) = u_A \left(\frac{SG_{\text{reference}} - 1}{SG_{\text{sample}} - 1} \right) \quad (7)$$

where $SG_{\text{reference}} = 1.020$ for Australian or US samples [10, 11], 1.015 for Bangladeshi samples [10] and other values are sometimes used [78]. It is often suggested that samples that are too dilute ($SG_{\text{sample}} < 1.010$) or too concentrated ($SG_{\text{sample}} > 1.030$) should be discarded [72], although this is not always done [11] and other limits are sometimes used [74]. For example, Gaines *et al.* [75] reported that 63 or 9 of 417 samples (17%) were too dilute ($SG < 1.010$) or concentrated ($SG > 1.030$), respectively (it is clear from the distributions reported by these authors that not all of these samples were also excluded based on u_{Cr}). Strongly nonlinear relationships between (i) u_{A} corrected by SG and that corrected by u_{Cr} [79–82] and (ii) u_{Cr} and SG [52, 75, 78] are often reported. Perhaps of even more relevance, it has been reported that SG-based estimates of J_{V} can be very unreliable [83].

3 The connection between blood and urine

The fluid and solutes present in urine are derived from the blood by filtration through the glomeruli in the kidneys. Two simple conservation relations relating to this were first formulated by Rehberg [84]. First, the volume conservation relation simply states that the volume of the blood filtered through the glomeruli (F) is matched by the total of the volumes of fluid that are reabsorbed (R) and excreted as urine (V) or

$$F = V + R \quad (8)$$

where, as Rehberg observed, R and F are much greater than V . Second, the material conservation relation just states that the quantity of analyte A filtered through the glomeruli (q_{A}) matches that in the urine if A is not reabsorbed

$$q_{\text{A}} = s_{\text{A}}F = u_{\text{A}}V \quad (9)$$

where s_{A} and u_{A} are the concentrations of A in blood and urine, respectively. Combining (8) and (9) yields

$$R = F - V = \left(\frac{u_{\text{A}}}{s_{\text{A}}} - 1 \right) V \quad (10)$$

from which, since R can not be negative, it follows that $u_{\text{A}} \geq s_{\text{A}}$, although u_{A} is actually much greater than s_{A} .

Differentiating the material conservation relation (9), and using (1), gives the instantaneous excretion rate of A

$$j_{\text{A}} = \frac{dq_{\text{A}}}{dt} = s_{\text{A}} \frac{dF}{dt} + F \frac{ds_{\text{A}}}{dt} = u_{\text{A}} \frac{dV}{dt} + V \frac{du_{\text{A}}}{dt} = \frac{du_{\text{A}}V}{dt} \quad (11)$$

However, the usual urinary measure is not instantaneous, because it takes a subject some time to accumulate sufficient urine to provide a sample. Instead, the urinary measure is the equivalent of the time average of the instantaneous excretion rate

$$\begin{aligned} \langle j_{\text{A}} \rangle &= \frac{1}{\Delta t} \int_0^{\Delta t} \frac{du_{\text{A}}V}{dt} dt = \frac{1}{\Delta t} \int_{u_{\text{A}}(0)V(0)}^{u_{\text{A}}(\Delta t)V(\Delta t)} du_{\text{A}}V \\ &= \frac{u_{\text{A}}(\Delta t)V(\Delta t) - u_{\text{A}}(0)V(0)}{\Delta t} = \frac{u_{\text{A}}V}{\Delta t} \end{aligned} \quad (12)$$

which is just J_{A} (12). In (12), $V(0)$ and $u_{\text{A}}(0)$ are, respectively, the preceding post-void residual volume and the concentration of A in that volume which are impractical to measure for a woman monitoring her own fertility. As we have argued previously [9], the post-void residual volume is much smaller than the void volume providing that Δt is sufficiently large. It is generally accepted that this is the case if $\Delta t \geq 3$ hours [5, 85]. Even ignoring this approximation, the practical reality is that a measurement of s_{A} reflects the situation at the time (and anatomical location) of sampling, whereas any urine measurement is averaged over the time (Δt) required to accumulate the volume of urine.

As $J_{\text{A}} = \langle j_{\text{A}} \rangle$ rather than j_{A} (12), it follows from (11) that

$$J_{\text{A}} = \left\langle s_{\text{A}} \frac{dF}{dt} + F \frac{ds_{\text{A}}}{dt} \right\rangle = \left\langle s_{\text{A}} \frac{dF}{dt} \right\rangle + \left\langle F \frac{ds_{\text{A}}}{dt} \right\rangle \quad (13)$$

but as the clearance (dF/dt) is usually considered to be constant [86]

$$J_{\text{A}} = \langle s_{\text{A}} \rangle \frac{dF}{dt} + \left\langle F \frac{ds_{\text{A}}}{dt} \right\rangle \quad (14)$$

This simply means that the time average of the instantaneous excretion rate (J_{A}) is not the same as the instantaneous serum measurement ($s_{\text{A}} dF/dt$) to which it is usually equated [86], especially if $ds_{\text{A}}/dt \neq 0$ which is usually the case for the reproductive hormones. In summary, the combination of (11) and (12) gives

$$J_{\text{A}} = \frac{u_{\text{A}}V}{\Delta t} \approx s_{\text{A}} \frac{dF}{dt} + \left\langle F \frac{ds_{\text{A}}}{dt} \right\rangle \quad (15)$$

where the approximation arises from the common assumption that it is legitimate to relate a time-averaged urine measurement (J_{A}) to an instantaneous serum meas-

urement (s_A). In effect, the implicit assumption is that s_A is a reasonable estimate of $\langle s_A \rangle$. Perhaps the most significant difference between (15) and the standard textbook expression [86] is that there is no assumption that $ds_A/dt = 0$. Instead, as $F > 0$, (15) indicates that if ds_A/dt averaged over Δt is not zero then the rate at which s_A changes contributes (positively or negatively) to J_A . (Note that $\langle Fds_A/dt \rangle \neq \langle F \rangle \langle ds_A/dt \rangle$ unless the covariance of F and ds_A/dt happens to be zero.) This is entirely consistent with the common practice of infusing with an analyte when measuring its clearance. In such circumstances (that is when $ds_A/dt = 0$) the standard textbook expression [86] applies. Conversely, it implies that where $ds_A/dt \neq 0$, as is generally the case for reproductive hormones, this is not the case.

4 Some implications

The theory we have outlined has several implications of which we explicitly consider six that are of particular significance to the monitoring of urinary analytes. First, the relationship between u_A and s_A is likely to be inconsistent because it depends on F and V , both of which are functions of time. Second, the standard measure of J_A is a time average of the instantaneous excretion rate (j_A) which is particularly significant for some analytes. Third, it suggests that there is a linear relationship between J_A and s_A , if and only if dF/dt is approximately constant as is usually assumed in the measurement of clearance. Fourth, the relationship between J_A and s_A has an intercept that is proportional to the time-averaged value of Fds_A/dt . Fifth, the equations (3, 6, 7) imply specific relationships between corrected u_A and J_V . We will consider the relationship between the uncorrected u_A and J_V elsewhere (Brown, Cooke and Blackwell, manuscript in preparation) so this aspect of the theory is omitted. Sixth, the kinetics of s_A and J_A are not necessarily related in any simple manner which has particular relevance to the analysis of the menstrual cycle. We discuss several examples in section 5.

4.1 The relationship between u_A and s_A

The material conservation relation (9) indicates that the urinary and serum concentrations of an analyte are not related in a simple manner. The relationship between u_A and s_A is positive, but inconsistent because it depends on F and V (9), both of which are functions of time. It is common knowledge that V changes depending on fluid intake, although there is a physiological limit to J_V , but that

there is little corresponding change in the blood [87, 88]. From this it follows that there is no good reason to expect a reasonable correlation between u_A and s_A for an individual subject, although good correlations have sometimes been reported for some analytes. It is the inconsistency in the correlation that is the fundamental problem: it is not possible to know when the correlation is reliable and when it is not. In effect, u_A provides an unreliable means of estimating s_A and if urinary analyte concentrations are monitored with that intention, as is sometimes the case in fertility monitoring, this should be made clear.

4.2 The excretion rate (J_A)

The standard measure of the excretion rate (J_A , (2)) is a time average of the instantaneous excretion rate (j_A). This tends to smooth the effects of both regular and sporadic fluctuations of s_A that would, in principle, be apparent in j_A if it could be measured conveniently. For example, the serum concentration of each of several reproductive hormones is known to vary systematically on at least two different time scales. First, their concentrations exhibit pulsatility on a time scale that tends to be small compared with the widely accepted minimum Δt of 3 hours [89-101]. This means that while s_A can depend significantly on where in the 'cycle of pulsation' the serum sample is obtained, J_A is averaged over at least three hours, thereby providing a more reproducible measure by minimising the effects of the rapidly changing s_A . Second, their concentrations vary systematically during the menstrual cycle [1, 8], rising or falling over several days (an important exception to this is luteinising hormone which usually rises very rapidly just before ovulation [102]). In such cases the time averaging over a few hours ($\Delta t \geq 3$ hours) that is associated with measuring urinary metabolites does not present any significant problem (Cooke, Blackwell and Brown, manuscript in preparation).

In other cases, the impact of specific events can be more or less transitory. For example, after eating meat the serum creatinine concentration (s_{Cr}) can rise and fall over several hours [57] and this may recur at the next meal or not for several days. While assaying the rate of creatinine excretion (J_{Cr}) does not eliminate this effect, the time averaging of j_{Cr} (12) reduces its amplitude to some extent. In contrast, deteriorating kidney function causes s_{Cr} to increase more slowly, over several months [103] or in transient cases it can rise or fall over days [104]. Similarly, some medications can increase s_{Cr} [105-107]. In all of these cases, the time averaging associated with the measurement of J_{Cr} is not a technical limitation.

4.3 The relationships between J_A and s_A (15)

The analysis indicates that there is a linear relationship between J_A and s_A (15) if the clearance (dF/dt) is constant as is usually assumed [86]. The same is not true of the relationship between J_A and u_A (Brown, Cooke and Blackwell, manuscript in preparation). This is unsurprising given the inconsistent relationship between u_A and s_A , but it is also consistent with the fact that J_V , the time averaged value of dV/dt , is not constant in most circumstances and nor is dV/dt [29]. This means that s_A is more reliably estimated using J_A rather than u_A , but, despite this, it should not be assumed automatically that $dF/dt = u_A V/s_A$ because ds_A/dt need not be zero (15).

4.4 Varying s_A (ds_A/dt)

The relationship between J_A and s_A has a non-zero intercept that is proportional to ds_A/dt . This means that (i) $dF/dt \neq J_A/s_A$ unless $ds_A/dt = 0$, consistent with the practice of ensuring this by infusion when measuring clearance, (ii) if ds_A/dt varies systematically, as it does for the reproductive hormones during the menstrual cycle, and this is not taken into account, there is systematic variation in the bias in the estimate of dF/dt or s_A , and (iii) the correlation between J_A and s_A can be good, but it need not be, and there is no reason to expect that it should be similar for several different experiments if ds_A/dt is inconsistent (Appendix 1).

Analysis of the data from some reports yields estimates of $\langle Fds_A/dt \rangle$ that are not statistically different from zero even if s_A is not constant. We consider that this reflects the ‘noise’ in the data because, as we show in section 5, high quality data obtained from experiments involving various analytes in which s_A changes consistently yield values of the intercept consistent with (15). We infer from this that our interpretation of (15) is reasonable.

4.5 Creatinine and specific gravity corrections of u_A

Comparison of (6), (7) and (3) yields at least two observations. First, that in each expression u_A is multiplied by a ‘correction factor’: J_V , $1/u_{Cr}$ or $f(SG)$ in (3), (6) and (7), respectively. Second, if each of these ‘corrected’ forms of u_A is a potentially viable means of monitoring s_A , then the three correction factors should be correlated in specific

ways. That is, (i) J_V should be inversely related to u_{Cr} , (ii) J_V should be linearly related to $f(SG)$ and (iii) $f(SG)$ should be inversely related to u_{Cr} . As we show below (section 5), without correction u_A is an unreliable means of monitoring s_A . If any of these relationships ((3), (6) and (7)) is unsatisfactory, then at least one of the ‘correction factors’ should be reconsidered. If any pair of relationships is unsatisfactory, then it is likely that the common ‘correction factor’ is flawed.

4.6 The kinetics of s_A and J_A are not necessarily simply related

During the menstrual cycle the levels of the reproductive hormones and their urinary metabolites change systematically, but they do not necessarily move perfectly in parallel. For example, Monfort *et al.* [108] report correlations (r) between serum E2 and creatinine-corrected urinary oestrone conjugate (E1C) concentrations ranging from -0.176 to +0.948 for 14 nonconceptive cycles. The mean correlation coefficient was 0.596, similar to the mean values in some reports [10, 109–111], but lower than those in some others [112–114]. However, when Monfort *et al.* [108] introduced a 24 h offset “to account for an approximately 24-h delay in excretion of urinary hormone metabolites”, the range became +0.465 to +0.945 (mean of +0.745). It will be apparent that this strategy does ‘improve’ the correlations, but at least one of them remains unconvincing.

In their analyses of short term experiments involving the ingestion or intravenous injection of various compounds, Dominguez and Pomerene [115] explicitly assumed that $s_A(t) = \kappa j_A(t)$, where t is time and $\kappa > 0$ is a constant. This implies that s_A and j_A change in parallel and, specifically, reach maxima at the same time. However, it follows from the material conservation relation (9) that the maxima of s_A and j_A do not coincide in this way if the clearance (dF/dt) is constant (Appendix 2). Intuitively, it seems unlikely that the maxima might coincide given that urine is derived from serum (section 3), implying differing kinetics. This is inconsistent with the assumption made by Dominguez and Pomerene [115], but it is consistent with the strategy employed by Monfort *et al.* [108] and others [10, 111]. Note, however, that while this idea might be written as $s_A(t) = \kappa u_A(t - \delta)/u_{Cr}(t - \delta) \approx \kappa J_A(t - \delta)$, where δ is the ‘offset’, it is yet to be established that κ (or κ') is constant rather than a function of ds_A/dt as might be anticipated given (15).

5 Some experimental examples

During the menstrual cycle the serum concentrations of reproductive hormones rise and fall successively [5, 8], so ds_A/dt changes from negative to positive and back during the cycle. To avoid this complexity, we consider data from experiments involving the excretion of exogenous creatinine [84, 116–119], urea [120, 121], inulin [122, 123], galactose and xylose [124]. Where appropriate, we distinguish between general and analyte-specific observations using the subscripts A (s_A , for example) and the relevant analyte (s_{Cr} , for example, in the case of creatinine), respectively.

5.1 Decreasing s_A ($ds_A/dt < 0$)

The rate of creatinine excretion (J_{Cr}) is approximately constant for an individual on a meat-free diet, but the rate differs between individuals [55, 125–128]. As would be anticipated, s_{Cr} increases following creatinine ingestion and then decreases again with a rate constant of about 0.17 h^{-1} as creatinine is excreted [118, 119, 129], consistent with the half lives reported by Chiou [130, 131]. The data indicate that it takes about 1–1.5 h to reach the maximum s_{Cr} after ingestion, although this can be greater if the dose is high [84, 116, 118, 119, 129, 132].

Rehberg [84, 116] carried out experiments on himself in which he ingested 5 g of creatinine in 200 mL of water and, starting about an hour later, periodically measured serum and urinary creatinine. He did not control his fluid or food intake and he carried on with his normal activities, so that there were considerable differences among the five experiments. The data show a monotonic decline in s_{Cr} that is consistent for all his experiments (Figure 1A). Despite the relative lack of control of the conditions, all the data can be described using a single exponential decay for which the rate constant is $k = 0.14 \pm 0.01\text{ h}^{-1}$ (95% CI). While the observed decline in s_{Cr} is expected (Figure 1A), there is no obvious pattern in the corresponding u_{Cr} data (Figure 1B). In contrast, J_{Cr} falls exponentially (Figure 1C) and $k = 0.15 \pm 0.02\text{ h}^{-1}$ (95% CI), which is not significantly different from that for the decline in s_{Cr} shown in Figure 1A ($p = 0.221$). Based on the difference in the patterns of the data in Figure 1A and 2B, it is not unexpected that the correlation between u_{Cr} and s_{Cr} is not strong ($r = 0.497$, $p = 0.001$, Figure 1D). In contrast, given that J_{Cr} and s_{Cr} decline similarly (Figure 1, A and C), it is unsurprising that there is a linear relationship between J_{Cr} and s_{Cr} ($\beta_0 = -89 \pm 87\text{ mg h}^{-1}$ (95% CI), $\beta_1 = 9 \pm 2\text{ L h}^{-1}$ (95% CI), $r = 0.881$, $p < 0.001$, Figure 1E). In this case the intercept is not significantly different from zero ($p = 0.053$), but because $ds_{Cr}/dt <$

0 (Figure 1A) β_0 should be negative if (15) is correct. Based on these estimates, we infer from (15) that the creatinine clearance (dF/dt) was 9 L h^{-1} and $\langle Fds_{Cr}/dt \rangle \approx -89\text{ mg h}^{-1}$ for Rehberg's data.

The fundamental features of Rehberg's results are not peculiar to humans or to creatinine. Among many possible examples, Figure 2 is a summary of the results obtained from a single experiment in which galactose was injected into a dog at $t = 0\text{ h}$ [133]. Both $s_{\text{galactose}}$ and $J_{\text{galactose}}$ decline exponentially (Figures 2, A and C), but no consistent pattern is apparent for $u_{\text{galactose}}$ (Figure 2B). It follows that there is no obvious relationship between $u_{\text{galactose}}$ and $s_{\text{galactose}}$ (Figure 2D), but there is a strong correlation between $J_{\text{galactose}}$ and $s_{\text{galactose}}$ (Figure 2E). These data are reminiscent of Rehberg's creatinine data (Figure 1) and similar canine data involving xylose [134] or creatinine [135]. Data from experiments involving rabbits injected with creatinine [136, 137] have been reported in less detail, but the relationship between J_{Cr} and s_{Cr} is just as clear.

It might be objected that these data were collected over just a few hours and might not be replicated over longer periods. However, Cope's [117] data are consistent with those of Rehberg [84, 116], but his experiments were more carefully controlled. In 21 separate experiments Cope [117] ingested a varying quantity of creatinine (up to a maximum of 7 g) and measured J_{Cr} between one and two hours after ingestion and s_{Cr} at the midpoint (one and a half hours after ingestion). Unlike Rehberg [84, 116], Cope refrained from fluid or food intake and was recumbent throughout each of the experiments. The latter factor is relevant because posture affects kidney function [138, 139] and normal daily activities can affect the consistency of the data obtained [120]. In Cope's data, the relationship between u_{Cr} and s_{Cr} is clearly nonlinear (Figure 3A), although it is positive ($r = 0.916$, $p < 0.001$) and is much clearer than that obtained by Rehberg (Figure 1D). However, a plot of J_{Cr} against s_{Cr} (15) has a slope ($=dF/dt$) of $12.7 \pm 0.5\text{ L h}^{-1}$ (95% CI) and an intercept ($=\langle Fds_A/dt \rangle$) of $-68 \pm 20\text{ mg h}^{-1}$ (95% CI) and is clearly linear ($r = 0.997$, Figure 3B). As $F > 0$, the negative intercept is consistent with a declining s_{Cr} ($ds_{Cr}/dt \leq 0$) at the time of measurement (about 1.5 h after creatinine ingestion). While it is not possible to confirm this because Cope measured s_{Cr} only once in the course of each experiment, it is a reasonable supposition based on Rehberg's data (Figure 1A) and several reports of similar experiments [118, 119, 129, 132]. For completeness, we point out that Cope [117] plotted a form of Figure 3B and, without the benefit of (15), regarded the non-zero intercept as an unexplained artefact arising from the determination of plasma creatinine.

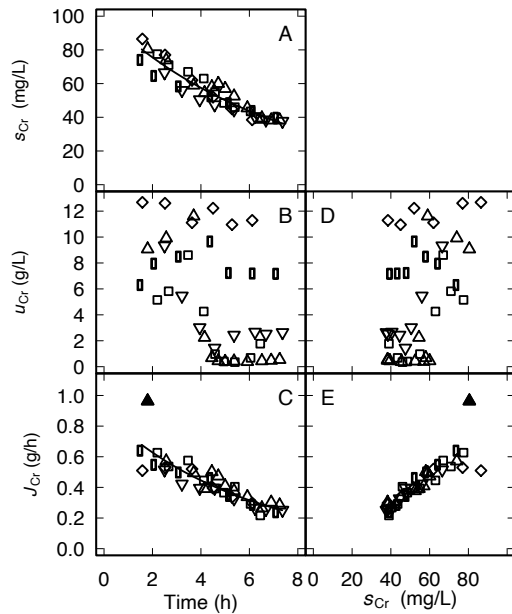


Figure 1: The (A) serum (s_{Cr}) or (B) urine creatinine concentration (u_{Cr}), (C) the creatinine excretion rate (J_{Cr}) observed following the ingestion of various quantities of creatinine, and the relationships between u_{Cr} (D) or J_{Cr} (E) and s_{Cr} . The curves in (A) and (C) are single exponential functions fitted by nonlinear least squares for which the rate constants (k) are $0.13 \pm 0.02 \text{ h}^{-1}$ (95% CI) and $0.17 \pm 0.04 \text{ h}^{-1}$ (95% CI), respectively, which are not significantly different ($p = 0.221$). In (E) the line is the least squares fit to the data ($r = 0.881$) and the slope ($\beta_1 = 9 \pm 2 \text{ L h}^{-1}$ [95% CI]) is significantly different from zero ($p < 0.001$), but the intercept ($\beta_0 = -0.09 \pm 0.09 \text{ g h}^{-1}$ [95% CI]) is not ($p = 0.057$). The data are those reported by Rehberg [84, 116] for a series of experiments he carried out on himself. Note that the outlier (\blacktriangle) was omitted from the analysis in (A), (C) and (E).

Addis and Drury [120] carried out similar experiments on themselves using urea. They monitored their serum and urinary urea levels starting at least three hours after the ingestion of a unspecified range of quantities of the solid dissolved in a litre of water. Other than drinking 500 mL of water each hour there was no other food or fluid intake during the course of the measurements. They repeated this for 27 and 31 days, respectively, and Drury obtained the data shown in Figure 4. In this case, the slope is $5.9 \pm 0.2 \text{ L h}^{-1}$ (95% CI) and the intercept is $-0.1 \pm 0.1 \text{ g h}^{-1}$ (95% CI), which is significantly different from zero ($p = 0.044$). Based on (15), we infer that the clearance of urea (dF/dt) was 5.9 L h^{-1} and that $\langle Fds_{\text{urea}}/dt \rangle = -0.1 \text{ g h}^{-1}$. For these data $r = 0.997$, but the corresponding data obtained by Addis are rather less convincing ($r = 0.982$), perhaps because he carried on with his normal activities during the experiments, and one effect of this is that the intercept is not significantly different from zero ($p = 0.380$).

Intravenous injection of an analyte yields an essentially instantaneous increase followed by a decline in s_A ,

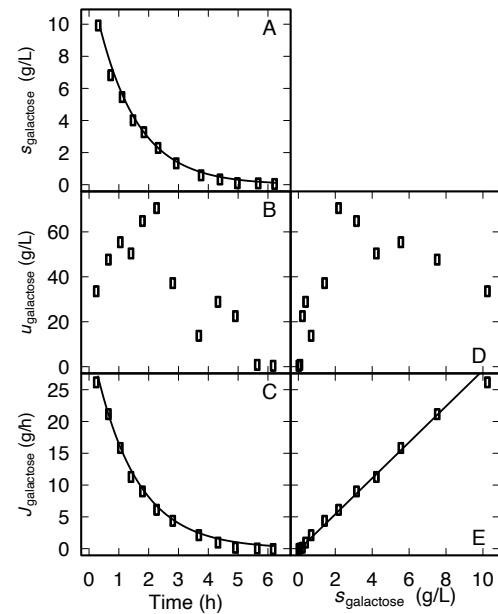


Figure 2: The (A) serum ($s_{\text{galactose}}$) or (B) urine galactose concentration ($u_{\text{galactose}}$), (C) the galactose excretion rate ($J_{\text{galactose}}$) observed following the injection of galactose, and the relationships between $u_{\text{galactose}}$ (D) or $J_{\text{galactose}}$ (E) and $s_{\text{galactose}}$. The curves in (A) and (C) are single exponential functions fitted by nonlinear least squares for which the rate constants (k) are $0.75 \pm 0.04 \text{ h}^{-1}$ (95% CI) and $0.71 \pm 0.05 \text{ h}^{-1}$ (95% CI), respectively, which are not significantly different ($p = 0.156$). In (E) the line is the least squares fit to the data ($r = 0.999$) and both the slope ($\beta_1 = 2.83 \pm 0.09 \text{ L h}^{-1}$ [95% CI]) and the intercept ($\beta_0 = -0.2 \pm 0.1 \text{ g h}^{-1}$ [95% CI]) are significantly different from zero ($p \leq 0.012$). The data are those reported by Dominguez and Pomeroy [133].

thereby eliminating any possibility of ambiguity about ds_A/dt . One example of this involves the injection of inulin [122, 140, 141] which is a non-metabolisable oligosaccharide [142]. The data of Ferguson and colleagues show that both the serum inulin concentration (s_{in}) and the inulin excretion rate (J_{in}) decline approximately exponentially after injection. For each subject J_{in} increases linearly with s_{in} ($r = 0.988$ – 0.999) and the intercept is negative [122]. These data are consistent with (15) given that $ds_{\text{in}}/dt < 0$.

We have shown that the relationship between u_A and s_A can be negligible (Figures 1D and 2D) or positive (Figure 3A), but even in laboratory conditions the relationship between u_A and s_A can be negative. For example, Shannon *et al.* [143] measured the excretion of xylose and creatinine. They show that as both s_{xylose} and s_{Cr} decline, the corresponding urinary concentrations actually increase (Figure 5, A and C) because the data are confounded by a corresponding decline in J_v . In contrast, J_{xylose} and J_{Cr} both increase linearly with their respective serum concentrations (Figure 5, A and C) because J_v is taken into account (15).

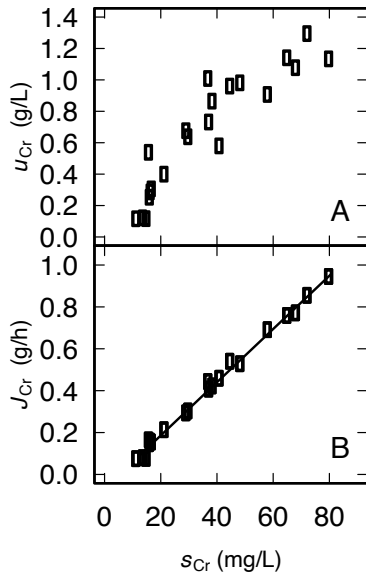


Figure 3: Relation between serum creatinine concentration (s_{Cr}) and (A) the urinary creatinine concentration (u_{Cr}) or (B) the creatinine excretion rate (J_{Cr}) observed following the ingestion of various quantities of creatinine. In (B) the line is the least squares fit to the data ($r = 0.997$) and each parameter ($\beta_1 = 12.7 \pm 0.5 \text{ L h}^{-1}$ [95% CI], $\beta_0 = -0.07 \pm 0.02 \text{ g h}^{-1}$ [95% CI]) is significantly different from zero ($p < 0.001$). The data are those reported by Cope [117] for a series of experiments he carried out on himself.

5.2 Constant s_A ($ds_A/dt = 0$)

In experiments in which a single bolus of a compound is ingested or injected, the serum concentration rises rapidly and then falls, but s_A is roughly constant ($ds_A/dt = 0$) for only a brief period. The time during which $ds_A/dt = 0$ can be prolonged by infusing a compound continuously or, in some circumstances, if the subject refrains from consuming any of that compound prior to and during the monitoring period.

It is standard practice to infuse continuously with inulin when measuring glomerular filtration rate and, in these circumstances, s_{in} can be maintained at approximately constant specific values (so that $ds_{in}/dt \approx 0$). In such experiments, consistent with (15), J_{in} depends linearly on s_{in} and the intercept (Fds_{in}/dt) is not significantly different from zero. For example, Kennedy *et al.* [123] carried out inulin infusion experiments on 14 subjects, for each of whom several different s_{in} were maintained. Their data indicate that the intercept of J_{in} versus s_{in} was not significantly different from zero for any of the subjects [123], consistent with $ds_{in}/dt = 0$ at each sampling time. Shannon and Smith [144] show similar data for a further four subjects. The intercept was not significantly different

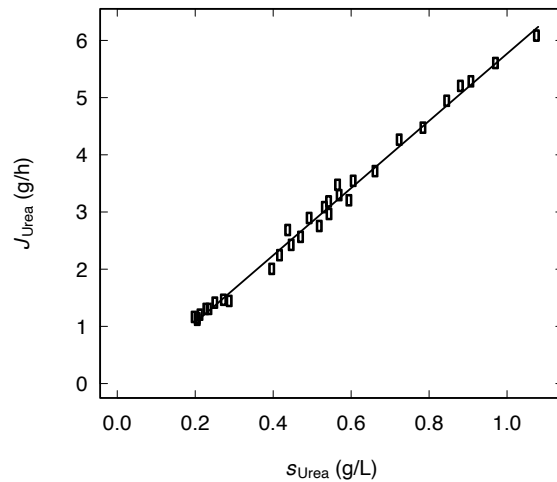


Figure 4: Relation between serum urea concentration (s_{urea}) and the rate of urea excretion (J_{urea}) for a series of urea ingestion experiments carried out on a single human subject [120]. The line is the least squares fit to the data ($r = 0.997$) and each parameter ($\beta_1 = 5.9 \pm 0.2 \text{ L h}^{-1}$ [95% CI], $\beta_0 = -0.1 \pm 0.1 \text{ g h}^{-1}$ [95% CI]) is significantly different from zero ($p < 0.001$ and $p < 0.044$, respectively). A second subject carried out the same series of experiments but carried on with his daily activities (unlike the subject from whom the plotted data were obtained) and in that case $r = 0.982$, $\beta_1 = 4.4 \pm 0.3 \text{ L h}^{-1}$ [95% CI] and $\beta_0 = 0.1 \pm 0.2 \text{ g h}^{-1}$ [95% CI] but the intercept is not significantly different from zero ($p = 0.380$).

from zero for any of them ($p \geq 0.112$, based on our digitisation of their data).

5.3 Increasing s_A ($ds_A/dt > 0$)

Equation (15) indicates that the intercept of J_A versus s_A should be positive when s_A increases throughout the experiment ($ds_A/dt > 0$). One report of experiments in which s_A is continuously increased while both blood and urine samples are collected is that of Drury [121]. He reported experiments in which dogs were infused continuously with urea at rates exceeding the urea excretion rate (J_{urea}). This resulted in a continuous increase in the serum urea concentration (s_{urea}) in samples collected over several hours. These data show that as s_{urea} increased, u_{urea} increased ($r = 0.967$; Figure 6A), decreased ($r = -0.597$; Figure 6B) or was approximately constant ($r = -0.051$; Figure 6C). In every case, J_{urea} increased linearly with s_{urea} (Figure 6, D-F) and the correlation coefficient (r) ranged from 0.952 to 0.991. In all cases except one (Figure 6E) the intercept was positive, consistent with $ds_{urea}/dt > 0$ (15), although in the single exception the intercept was signifi-

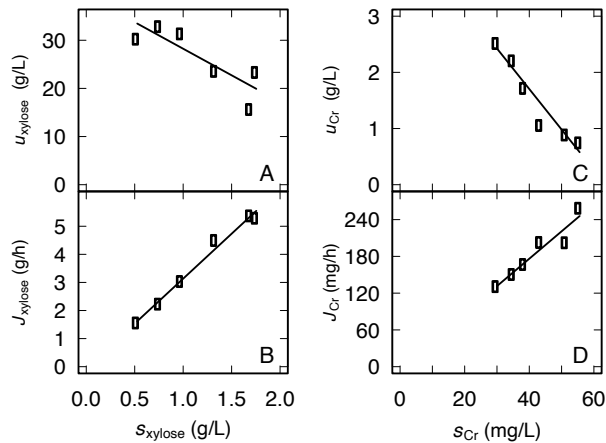


Figure 5. Relation between the serum concentration of xylose (s_{xylose}) or creatinine (s_{cr}) and the corresponding urinary concentrations (u_{xylose} (A) and u_{cr} (C)) and excretion rates (J_{xylose} (B) and J_{cr} (D)) for an experiment carried out on a dog [143]. In each panel the line is the least squares fit to the data ($r = -0.850$ and -0.958 for (A) and (C), respectively, and $r = 0.993$ and 0.956 for (B) and (D), respectively).

cantly different from zero ($p = 0.012$). This experiment provides a further support for (15).

The material conservation relation (9) indicates that the relationship between the urinary and serum concentrations of an analyte is not a simple one. Specifically, there is no good reason to assume that u_A and s_A are linearly related (Figures 1D and 2D, although they may be positively correlated). The results of Cope's [117] creatinine ingestion experiments illustrate this point (Figure 3A). However, (11) does lead to the prediction that the creatinine excretion rate (J_A) is linearly related to s_A (15), although the intercept does vary depending on the rate of change of s_A . Again, Cope's [117] data conform to this pattern (Figure 3B) in that it is likely that $ds_A/dt \leq 0$ in his experiment [118], although his data do not permit confirmation of this, and the estimate of Fds_A/dt derived from his data is negative (Figure 3B). Cope's [117] data provide clear justification for the use of the excretion rate (J_A) rather than the urinary concentration (u_A) to estimate s_A even in very tightly controlled circumstances. In less well controlled experiments (Figures 1 and 2) and presumably in the lives of most women [145], there is even less justification for relying on u_A rather than J_A .

5.4 Comparing u_A and s_A

It is clear from (15) that u_A can be an unreliable means of monitoring s_A (Figures 1D and 2D) because, while $J_A = u_A J_V$ is proportional to s_A , J_V is not constant. The relation-

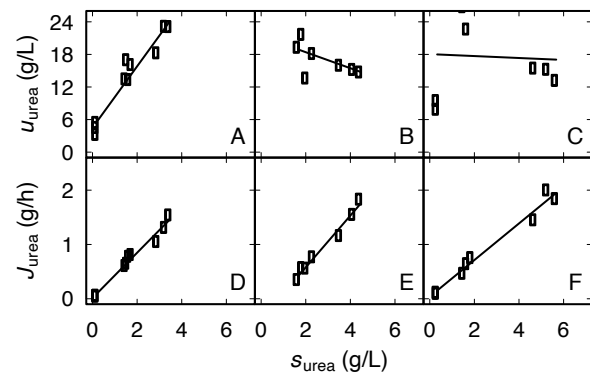


Figure 6. Relation between serum urea concentration (s_{urea}) and (A-C) urinary urea concentration (u_{urea}) and (D-F) the urea excretion rate (J_{urea}) for three of the five experiments (each with a different rabbit) reported by Drury [121]. In each experiment the rate at which urea was infused exceeded J_{urea} so that $ds_{\text{urea}}/dt > 0$. In each panel the line is the least squares fit to the data ($r = 0.967$, -0.597 and -0.051 for (A), (B) and (C), respectively, and $r = 0.989$, 0.989 and 0.988 for (D), (E) and (F), respectively).

ship can be slightly more reliable if the data are collected in very tightly controlled conditions (as, for example, in Figure 3), because daily activities affect J_V [120, 138, 139]. However, even in laboratory conditions, the relationship between u_A and s_A can be negative (Figure 5, A and C). In all of the examples we give, the corresponding correlation between J_A and s_A is strongly positive (Figures 1E, 2E, 3B, 5B and 5D). Equation (15) indicates that the critical difference is that J_V is considered because $J_A = u_A J_V$. The most significant problem for the use of u_A in monitoring s_A is inconsistency: while it may sometimes appear that u_A relates linearly to s_A , it takes relatively small fluctuations in J_V to destroy the illusion and even if J_V might be approximately constant for given subject for some time, it would be unwise to expect that the same relationship applied to different subjects.

5.5 Comparison of u_A correction factors

We have shown that s_A is more reliably monitored using J_A than u_A in even the most controlled circumstances (Figures 1, 2 and 3). However, J_A is merely u_A corrected using J_V (3), so it is reasonable to ask whether u_A corrected using u_{cr} (6) or $f(\text{SG})$ (7) might be just as useful. Viewed in this way, (i) u_{cr} should depend hyperbolically on J_V (3), (ii) $f(\text{SG})$ should be linearly related to J_V (7) and (iii) u_{cr} should depend hyperbolically on $f(\text{SG})$ ((6) and (7)). To test these predictions, we use the data based on 24 hour urine samples obtained over several days from subjects

on a constant intake of fluid and food [53]. Folin reported u_{cr} , V and SG , among other variables, and from these J_v , J_{cr} and $f(SG)$ can be calculated.

There is a clear inverse relationship between u_{cr} and J_v (Figure 7A) consistent with (3). However, as Folin [55] observed, despite all subjects having the same diet and fluid intake, J_{cr} varied among the subjects. Sufficient data were available to estimate J_{cr} for 33 subjects for whom the rates were approximately normally distributed (mean = 1.2 ± 0.1 g/24 h, SD = 0.30 ± 0.07 g/24 h) and ranged from 0.87 g/24 h to 1.51 g/24 h (the lower and upper bounds, respectively, of the shaded region in Figure 7A). It is apparent from these data that u_{cr} can change significantly with very small changes in J_v (the open squares in Figure 7A) or change very little despite relatively large changes in J_v (the open diamonds in Figure 7A). In these subjects J_v ranged from 0.3 to 3.075 L/24 h (equivalent to 13–128 mL/h), which is more limited than the 10–200 mL/h we have reported for overnight urine samples from women free to eat and drink *ad libitum* [29].

The relationship between $f(SG)$ and J_v is not particularly clear for the 44 of Folin's [53] subjects for whom sufficient data are available (Figure 7B). In some cases there is an essentially linear increase in $f(SG)$ as J_v increases, in other cases the relationship is nonlinear, but in five cases the relationship is negative so the correlation coefficient (r) ranges from -0.695 to 0.998. Overall, the slope ($df(SG)/dJ_v$, which has units of h/L) is approximately normally distributed (mean = 0.4 ± 0.1 h/L, SD = 0.37 ± 0.08 h/L) and ranges from -0.69 to 1.27 h/L.

The relationship between $f(SG)$ and u_{cr} is also somewhat unclear as is apparent from the inter-subject variation in the data shown in Figure 7C. Equations (6) and (7) imply that $f(SG)$ declines hyperbolically with increasing u_{cr} ($f(SG) \propto 1/u_{cr}$). However, rather than asymptotically approaching zero as u_{cr} rises, $f(SG)$ tends towards about 0.6 (Figure 7C). This means that a slightly more suitable empirical relationship is $f(SG) \approx \alpha_0 + (\alpha_1/u_{cr})$, where $\alpha_0 = 0.6 \pm 0.5$ (SD) and $\alpha_1 = 0.6 \pm 0.6$ (SD) are approximately normally distributed for the 33 subjects for whom sufficient data were reported [53] to permit their estimation. The uncertainties associated with these estimates are very large, consistent with the considerable inter-subject variation in the $f(SG)$ - u_{cr} relationship. Nevertheless, the value of α_0 is consistent with the normal range of urinary SG, which is 1.008 to 1.030 [146], although values as low as 1.001 and at least as high as 1.044 have been reported [146, 147]. Nevertheless, $f(1.030) = 0.67$, which is not significantly different from the asymptote estimated from Folin's data (nor does the asymptote differ from $f(1.044) = 0.45$).

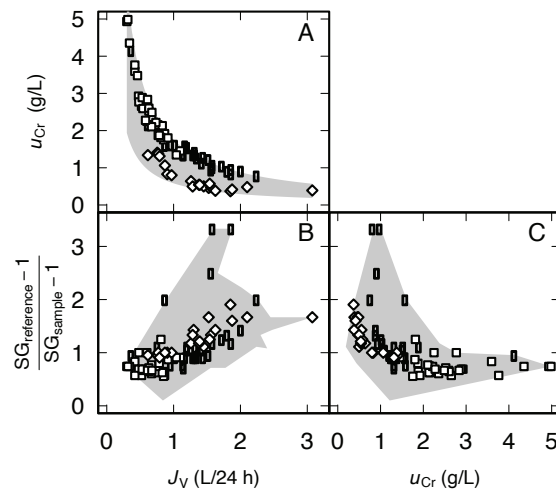


Figure 7. Relation between (A) urinary creatinine concentration (u_{cr}) and urine production rate (J_v) and between $f(SG)$ and J_v (B) and u_{cr} (C) for each of three different subjects (consistently identified in each panel: ●, ◇, □) maintained on a constant diet. In each panel, the grey zones are concave hulls constructed around the all the available data and are only intended to give an indication of the range of variation. The data are those reported by Folin [53].

6 Discussion

Changes in the serum concentration of an analyte (s_A) can be monitored by measuring the analyte or its metabolites in the urine. The most frequently used alternatives for measuring urinary analytes are the concentration (u_A , (2)), u_A corrected using either the creatinine concentration (u_A/u_{cr} , (6)) or specific gravity ($u_A f(SG)$, (7)), and the excretion rate (J_A , (3)), which can be considered to be u_A corrected using the urine production rate (J_v). We have used simple examples as the basis for a consideration of the usefulness of these measures of urinary analytes.

The analysis indicates that J_A is linearly related to s_A providing that the clearance (dF/dt) is constant (15). As J_A is the product of u_A and J_v (3) and J_v is not constant, there is no reason to expect that J_A should necessarily be systematically related to u_A . It follows that s_A and u_A are not necessarily related in any particular way either. The data show that J_A depends linearly on s_A (Figures 1E, 2E, 3B, 4B, 4D, 5B, 5D, 6D-F), consistent with (15), whether ds_A/dt is positive (Figure 3A), negative (Figure 4) or zero (Figure 6) as s_A increases. In contrast, the corresponding values of u_A show no apparent relationship (Figures 1D, 2D and 6C), or a positive (Figures 3A and 6A) or negative (Figures 5A, 5C and 6B) correlation with s_A . Certainly, the systematic decline of s_A in Figures 1A and 2A is not apparent in the corresponding u_A data (Figures 1B and 2B). This variability

of the u_A - s_A relationship is the essence of the problem in any analysis of the menstrual cycle based on u_A : from one day to the next a change in u_A might follow, diametrically oppose or be insensitive to changes in s_A , and one can not tell which of these possibilities applies. It is the systematic changes in the levels of reproductive hormones that are the basis of any reliable analysis of the menstrual cycle [1, 5, 8]. We have shown or cited examples that demonstrate that this is the case for at least creatinine, inulin, xylose, galactose and urea in humans, dogs and rabbits, but we anticipate that it also applies to other analytes and other species. The implication of the analysis, confirmed by these data, is that J_A is a reliable means of monitoring s_A , but u_A is not.

As J_A can be seen as u_A corrected by J_v , it is reasonable to ask whether J_A differs in any material way from other corrected forms of u_A : u_A/u_{Cr} and $u_A f(SG)$. Equations (3), (6) and (7) imply that (i) $u_{Cr} \propto 1/J_v$, (ii) $f(SG) \propto J_v$ and (iii) $f(SG) \propto 1/u_{Cr}$. Only the first of these relationships (3) is unambiguously supported by creatinine excretion data obtained from hospitalised subjects on a constant meat-free diet and exactly the same daily fluid intake [53]. Even in these data, however, J_{Cr} differs between subjects (Figure 7A). The other two relationships involve $f(SG)$ (Figure 7, B and C) and are much less convincing than that between u_{Cr} and J_v (Figure 7A). One of the difficulties with $f(SG)$ is that the range of urinary SG is limited [146] and so as u_{Cr} increases $f(SG)$ tends towards 0.6 (Figure 7C) rather than 0. This means that $f(SG)$ might be reasonably sensitive at low u_{Cr} , but is completely insensitive for $u_{Cr} > 1.5$ g/L (Figure 7C). As the normal range (10-90th percentiles) of u_{Cr} is 0.3354 to 2.372 g/L for north American subjects [148], we infer from this that $f(SG)$ is likely to be essentially constant for a significant proportion of 'normal' subjects for whom u_{Cr} is changing. In this respect, u_A corrected using specific gravity (7) is likely to be less reliable than u_A corrected using u_{Cr} (6). This confirms what has been shown previously by various authors [52, 75, 78-82].

If J_v and u_{Cr} are merely factors used to 'correct' u_A and both vary between and within subjects, is there any significant difference between them? Given (3) and (12), and recalling that $E(Y)/E(X) = E(Y/X) + [1/E(X)]\text{cov}(Y/X, X)$ [149, 150] it follows that

$$\frac{u_A}{u_{Cr}} = \frac{\langle j_A \rangle}{\langle j_{Cr} \rangle} = \left\langle \frac{j_A}{j_{Cr}} \right\rangle + \frac{\text{cov}(j_{Cr}, j_A/j_{Cr})}{\langle j_{Cr} \rangle} \quad (16)$$

where $\text{cov}(\cdot, \cdot)$ is the covariance of the arguments. Recalling that $J_{Cr} = \langle j_{Cr} \rangle = u_{Cr} J_v$ and rearranging (16) yields

$$J_v = \left(\frac{\langle j_A \rangle - \text{cov}(j_{Cr}, j_A/j_{Cr})}{\langle j_A/j_{Cr} \rangle} \right) \frac{1}{u_{Cr}} \quad (17)$$

which means that (5) applies if j_{Cr} is literally constant, in which case $j_{Cr} = J_{Cr}$ and $\text{cov}(j_{Cr}, j_A/j_{Cr}) = 0$, but otherwise $1/u_A$ may or may not be related consistently to J_v . However, it is apparent from Folin's [53] data (Figure 7) and the literature [60-71, 125-127, 151-157] that J_{Cr} is not literally constant. Given this, the pragmatic approach would be to assume that the relationship between the creatinine-corrected u_A and J_A is not consistent because it depends on j_A and j_{Cr} (16), both of which may vary. The creatinine-corrected u_A yields neither (i) a direct relationship with s_A nor (ii) proportionality to J_A unless J_{Cr} is constant, which seems unlikely in normal daily life [60-71, 125-127, 151-157].

In addition to the linear J_A - s_A relationship, (15) indicates that there is second term that applies if s_A is not constant ($ds_A/dt \neq 0$). Specifically, (15) indicates that this term is negative, positive or zero if s_A is decreasing, increasing or constant, respectively. We have shown that high quality data are often consistent with this simple interpretation (Figures 1-6). The significance of this term in (15) is that s_A is probably rarely constant because of diet, exercise, damage to the kidney and very many other factors. For the reproductive hormones, which change systematically on several timescales, $ds_A/dt \neq 0$ for most of the menstrual cycle (an exception to this might be the P and PdG serum concentrations both of which are relatively constant from one day to the next during the follicular phase).

It might seem to some readers that we have dealt at some length with something that is well understood. After all, in 1957 Wray [158: 529] observed that "[t]he practice of reporting pregnanediol outputs in mg per unit volume is contra-indicated by its great inaccuracy" and very similar comments were made a century earlier by Thudichum [159]. Despite this, the use of u_A in the measurement of urinary metabolites of reproductive hormones continues [2, 6, 7, 30-46]. Moreover, there is still confusion in the literature about u_A , J_A and their link to J_v . For example, J_A and u_A have been compared directly without regard to their dimensional incompatibility and practical differences [160, 161] and, perhaps even more confusing, data reported as $u_A/\Delta t$ have been described as excretion rates [162] or concentrations [36]. We have not searched exhaustively for examples of this sort, but expect that there are more. We infer from this that while at least some of the theory we have outlined may be familiar, it is clearly not always understood.

7 Conclusions

We draw two main conclusions from the theory and the supporting examples we have given. First, urinary analyte concentration (u_A) does not provide a reliable estimate of either the serum analyte concentration (s_A) or the analyte excretion rate (J_A). The commercial devices that purport to monitor fertility and/or the menstrual cycle by following the urinary metabolites of reproductive hormones (or the hormones themselves) are currently based on measurements of u_A [163, 164]. Substantiation of the claims made about at least some of these is based on questionable interpretations of the relevant data [25]. Women and their partners deserve better than the rough guess available to them at present. Second, the creatinine-corrected analyte concentration provides a better indication of changes in the serum analyte concentration than u_A , but it can be unreliable if the rate of creatinine excretion is not constant. The real problem is that it is not necessarily possible to distinguish those times when the method works well from those when it does not. The most reliable and useful measure of a urinary analyte is the excretion rate (J_A).

Conflict of interest: DC is currently employed by Science Haven Limited, and LB is and SB has been a consultant to the same company. Science Haven Limited is developing assays for monitoring the menstrual cycle.

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Appendix 1

It follows from (15) that if s_A is falling ($ds_A/dt < 0$) then $J_A < s_A dF/dt$ and if s_A is rising ($ds_A/dt > 0$) then $J_A > s_A dF/dt$. More generally, where (15) applies, J_A should increase approximately linearly with s_A , providing both the clearance (dF/dt) and Fds_A/dt are approximately constant. In these circumstances, the relationship should have a slope of dF/dt and an intercept of $\langle Fds_A/dt \rangle$. If (15) is fitted to the data by ordinary least squares regression, then the expected value of the slope is $E(dF/dt) = rS_{JA}/S_{sA}$ [165] and that of the intercept is $E(Fds_A/dt) = \langle J_A \rangle - E(dF/dt)\langle s_A \rangle$, where $\langle x \rangle$ and S_x and are the mean and standard deviation of x , respectively, and r is the correlation coefficient between J_A and s_A . From this and (15)

$$r = \frac{CV_{sA}}{CV_{JA}} \left(1 - \frac{1}{\langle J_A \rangle} E \left(F \frac{ds_A}{dt} \right) \right) = \frac{S_{sA}}{S_{JA}} E \left(\frac{dF}{dt} \right) \quad (A1.1)$$

where $CV_x = S_x/\langle x \rangle$, which means that the correlation (r) between J_A and s_A will tend to be larger or smaller if $ds_A/dt < 0$ or $ds_A/dt > 0$, respectively. Similarly, r will tend to

increase (decrease) as S_{sA} rises (falls) or as S_{JA} falls (rises), so the ranges of variation of s_A and J_A determine whether there is or is not a statistically significant relationship between s_A and J_A . We accept that the least squares approach implies that the uncertainty in s_A is negligible, but a similar, albeit more complex, result is obtained when this is not the case using Deming regression, for example. While the use of r in contexts such as this is deprecated for good theoretical reasons [166], it continues to be used and (A1.1) may account for some of the variability in the reported correlations between the serum concentration and urinary levels of reproductive hormones [10, 11, 108, 110-114, 167] for which ds_A/dt may be zero, positive or negative. Based on (15), the usual clearance expression ($s_A dF/dt = J_A$) [86] is correct *only* when $ds_A/dt = 0$ (because $F > 0$). This is consistent with the standard practice in the measurement of clearance which is to ensure that $ds_A/dt = 0$ by infusing A [168, 169, p. 39-62]. It is not reasonable to assume that this is the case for reproductive hormones except, perhaps, for P in the follicular phase of the menstrual cycle. Following ovulation, for example, serum E2 falls and then both E2 and P transiently rise during the luteal phase of the cycle.

Appendix 2

The instantaneous excretion rate and s_A can not both be maximal at the same time if the clearance (dF/dt) is constant, which Dominguez and Pomerene [115, 118] assume. To see this recall from the material conservation relation (9), that the instantaneous excretion rate (j_A) is

$$\frac{dq_A}{dt} = j_A = s_A \frac{dF}{dt} + F \frac{ds_A}{dt} \quad (\text{A2.1})$$

(11) and so the maximal instantaneous excretion rate occurs when

$$\frac{dj_A}{dt} = s_A \frac{d^2F}{dt^2} + F \frac{d^2s_A}{dt^2} + 2 \frac{ds_A}{dt} \frac{dF}{dt} = 0 \quad (\text{A2.2})$$

However, (i) if the clearance ($dF/dt > 0$) is constant, then $d^2F/dt^2 = 0$, and (ii) $F > 0$, so

$$F \frac{d^2s_A}{dt^2} = -2 \frac{ds_A}{dt} \frac{dF}{dt} \quad (\text{A2.3})$$

when the instantaneous excretion rate is maximal. If s_A is also maximal at this instant, then $ds_A/dt = 0$ and $d^2s_A/dt^2 \neq 0$, which is inconsistent with (A2.3). It follows that $ds_A/dt \neq 0$ at the excretion rate maximum.