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Article *in* Journal of Theoretical Biology · October 1995

DOI: 10.1006/jtbi.1995.0176 · Source: PubMed

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J. theor. Biol. (1995) **176,** 59–65

Physiological Time and the Measurement of Glomerular Filtration Rate

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(*Received on* 30 *August* 1994, *Accepted in revised form on* 25 *April* 1995)

Diverse physiological timescales in different animals can have an important influence on the measurement of biological variables. In vertebrates, the absolute and relative rates of glomerular filtration span several orders of magnitude. These differences have important implications for the logistics of measurement protocols in different species. A simple model is used to examine the importance of physiological time to the measurement of glomerular filtration rate.

1. Introduction

The term physiological time describes the observation that different animals operate in an array of relative time scales (Calder, 1984). This relative time is manifested in virtually all aspects of an organism's existence, from expected lifespan to the time of a single cardiac cycle (Calder, 1984). Because these relative timescales so profoundly influence an animal's biology, the importance of physiological time to the application of measurement techniques also deserves consideration. Physiological measurements are performed on a variety of species that may differ greatly in body mass and phylogenic group. Two of the most important predictors of physiological time are body mass and phylogenic group (Calder, 1984). These differences should be recognized, and perhaps alternate measurement techniques should be applied in measuring physiological variables. The importance of physiological time should, therefore, be considered when initiating measurements on animals of different size or phylogeny. What follows is an examination of physiological timescale with respect to the measurement of glomerular filtration rate (GFR).

Glomerular filtration is a fundamental element in the processes that lead to osmotic homeostasis in vertebrates (Dantzler & Braun, 1980; Yokota *et al.*, 1985). The rate of filtration governs the amount and composition of fluid that will enter the nephron for processing. This central role of GFR in fluid homeostasis makes these measurements an important component in assessing and investigating renal function. In addition, GFR measurements may also be important to questions relating to other aspects of vertebrate biology such as ecology and energetics (see Beuchat *et al.*, 1991; Roberts, 1991).

The measurement of GFR in animals differing in phylogeny or body mass offers certain challenges that are not always obvious. Differences in physiological time mean that the percent of total plasma volume filtered per unit time differs drastically between different animals. This relationship arises because absolute filtration rates differ greatly between animals of different body size and phylogeny (Calder & Braun, 1983; Yokota *et al.*, 1985), while plasma volume tends to be a constant portion of body mass (Calder, 1984). GFR measurements on birds have been made on species spanning over three orders of magnitude in body mass. A 25 g bird has a GFR of about 0.20 ml min^{-1}, while a 1 kg bird has a GFR of about 2.35 ml min^{-1} (Williams *et al.*, 1991). However, the relative amount of plasma filtered is higher in the smaller animal since the total blood volume is equal to about 8% of body mass in mammals and birds (Calder, 1984). Assuming a hematocrit of around 40%, plasma volume is roughly 5% of an animal's total body mass. Thus, the 25 g bird will filter its entire plasma volume in just over 6 min. The larger 1 kg bird will require 21 min to filter the same proportion of its plasma. There are also large differences between phylogenic

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TABLE 1
GFR (ml hr^{-1}) as a function of body mass (g) in different vertebrate classes

Class	GFR (ml hr^{-1})	GFR (ml hr^{-1}) of 100 g animal	Number of species	Source
Mammals	1.24 M$^{0.765}$	42	41	Yokota *et al*., 1985
Birds	1.29 M$^{0.68}$	30	15	Williams *et al*., 1991
Reptiles	0.0058 M$^{0.999}$	0.58	22	Yokota *et al*., 1985
Amphibians	0.049 M$^{0.894}$	2.8	13	Yokota *et al*., 1985
Teleosts	0.010 M$^{0.788}$	0.38	15	Yokota *et al*., 1985

groups. Take for example, the differences between a 100 g mammal, bird, and reptile all having about a 5 ml plasma volume. The mammal will have a GFR of 0.70 ml min^{-1} (Yokota *et al*., 1985); the bird will have a GFR of 0.49 ml min^{-1} (Williams *et al*., 1991); and the reptile will filter 0.01 ml min^{-1} (Yokota *et al*., 1985). The time required to filter the total plasma volume will be 7 min for the mammal, 10 min for the bird, and 8.6 h for the reptile. These differences will create disparities in the dynamics of the variables involved in the measurement of GFR.

2. Relative Filtration Rate

The percent of total plasma volume filtered every minute will be referred to as the relative filtration rate (RFR). RFR can be measured directly or derived using estimates of GFR and estimates of total plasma volume. GFR can be estimated by using equations which describe absolute GFR as a function of body mass.

The relationships between GFR and body mass have been derived from selected species within the vertebrate classes (Table 1). GFR, as many functions, correlates with body mass in a logarithmic fashion:

$$GFR = aM^b. \quad (1)$$

Assuming that total plasma is 5% of body mass in all animals, and that the plasma has approximately the same density as water (1 g ml^{-1}), then RFR (% min^{-1}) can be related to body mass as:

$$RFR\ (\%/\text{min}^{-1}) = [GFR\ (\text{ml min}^{-1})/0.05\ \text{M (ml)}]\cdot 100\%. \quad (2)$$

By substituting the appropriate equations from Table 1, general relationships between RFR and body mass within a group of animals can be calculated (Table 2).

Note the differences in RFR which exist between animals of different body mass and phylogenic group. In all groups except for the reptiles, the exponential term is considerably less than zero (Table 2). A 1 kg chicken is predicted to have an RFR of 4.7% min^{-1}, while a 5 g hummingbird should have an RFR of 25.7% min^{-1}. Ectothermic vertebrates can be expected to have an RFR < 1, while most all mammals and birds will have an RFR > 1. The physiological significance of the differences in GFR between vertebrate groups is discussed elsewhere (Calder & Braun, 1983; Yokota *et al*., 1985). The importance for measurement purposes is that when RFR differs significantly between animals, the technical variables needed for GFR measurement must be adjusted accordingly.

3. Measurement of GFR

The determination of GFR involves the measurement of clearance of a filtration marker from the plasma. Clearance of a filtration marker is the volume of plasma completely cleared of the material per unit time. Clearance is described by the equation:

$$\mathring{V}_c = (C_u \cdot \mathring{V}_u)/C_p. \quad (3)$$

In this equation, C_u and C_p are the urinary and plasma concentrations of the fluid marker, respectively, and $\mathring{V}_u$ is the urinary flow rate (Brenner *et al*., 1986). If the marker is uncharged biologically inert, freely filtered at the glomerulus, and if the substance is neither secreted nor reabsorbed by the nephron, then the clearance of that marker is equal to the GFR (Brenner *et al*., 1986). While inulin is considered to be one useful filtration marker, there are a variety of other available compounds (Brenner *et al*., 1986).

The product of urinary marker concentration (C_u) and urinary flow rate ($\mathring{V}_u$), is equal to the marker

TABLE 2
RFR (% min^{-1}) as a function of body mass (g) in different vertebrate classes

Class	RFR (% filtered min^{-1})	RFR of 100 g animal
Mammals	41 M$^{-0.235}$	13.9
Birds	43 M$^{-0.32}$	9.9
Reptiles	0.19 M$^{-0.001}$	0.2
Amphibians	1.63 M$^{-0.106}$	1.0
Teleosts	0.33 M$^{-0.211}$	0.1

excretion rate ($\mathring{V}_{ex}$). This means that GFR is equal to $\mathring{V}_{ex}/C_p$. The dynamics of these two variables during a clearance period are largely determined by RFR. For example, the length of time from introduction of a plasma marker to the time of plasma marker stability, as well as the maintenance of such stability, are intimately related to RFR.

A simple mathematical model can be consulted to provide quantitative guidelines for measurements. The model also provides a conceptual framework for the measurement of GFR in animals of different body mass and phylogeny.

4. Mathematical Model

As a filtration marker is introduced into the circulation, the concentration in the plasma will begin to increase. At the same time, the substance will begin to be filtered at the glomerulus. The plasma concentration will increase until the rate of introduction and rate of elimination are equal. Once introduction rate and excretion rate are equal, the system will be in equilibrium until at least one of these rates changes. If either the introduction or excretion rate does change, then the system will reestablish a new equilibrium after a period of time. The length of that period is directly related to the RFR. If the RFR is high, then equilibrium will be established more quickly than if RFR is low.

Each minute the amount of the marker in the plasma, and therefore the plasma marker concentration, will be determined by four variables. These variables are the amount of marker introduced into the plasma during that minute, the amount of marker excreted during that minute, the amount of marker left in the circulation from the previous minute, and the total plasma volume (P_v). In turn, excretion rate will be the product of the total amount of marker in the plasma and the RFR. While changes in these variables actually occur continuously, it is convenient to break these processes into discrete events for modelling purposes. This means that logarithmic processes will be broken into a series of minute-by-minute linear steps. An example may best illustrate the basic nature of these relationships.

Assume an introduction rate of 1000 units of marker/min^{-1}, a plasma volume of 1 ml, and an RFR of 10% min^{-1}. During the first minute, 1000 units are introduced into the system. Before any marker is filtered, the plasma concentration is 1000 units ml^{-1}. After 10% of the marker is filtered, the total marker in the system is 900 units and the concentration is 900 units ml^{-1}. During the second minute, another 1000 units are introduced into the system and the total marker in the system is now 900 + 1000 or 1900 units. After filtration of 10% of the marker, 1710 units are left in the system. The amount of marker in circulation will increase each minute, until the excretion rate equals the introduction rate ($\mathring{V}_i$). The total amount of marker in the plasma (R_{total}) is defined by:

$$R_{total}(\text{units}) = \mathring{V}_{ex}(\text{units min}^{-1})/RFR(\%\ \text{min}^{-1}). \quad (4)$$

So once equilibrium is reached, the excretion rate will be 1000 units min^{-1}, and the amount of marker in the plasma will be 1000 (units min^{-1})/10% (min^{-1}), or 10 000 units.

The general format of this model system was written as a simple algorithm to increase the efficiency of the calculations. In this algorithm, the amount of marker in the circulation is defined in terms of three variables: the amount of marker in circulation at minute $x(R_x)$ can be defined in terms of the amount of marker in circulation at minute $x-1(R_{x-1})$; the amount of marker introduced into the system during minute $x(I_x)$; and the amount of marker excreted in minute x (E_x):

$$R_x(\text{units}) = R_{x-1}(\text{units}) + I_x(\text{units}) - E_x(\text{units}). \quad (5)$$

The amount of marker excreted during minute x is defined by:

$$R_x(\text{units}) \cdot RFR\ (\%\ \text{min}^{-1}). \quad (6)$$

During the first minute of our example:

$$R_1(\text{units}) = R_0(\text{units}) + I_1(\text{units}) - E_1(\text{units})$$

or

$$900(\text{units}) = 0(\text{units}) + 1000(\text{units}) - 100(\text{units}).$$

During the second minute:

$$R_2(\text{units}) = R_1(\text{units}) + I_2(\text{units}) - E_2(\text{units})$$

or

$$1710(\text{units}) = 900(\text{units}) + 1000(\text{units}) - 190(\text{units}).$$

A program written in BASIC implements this algorithm on a minute-by-minute basis. The figures and equations in the following discussion were generated using this fundamental program. The only assumptions in this model are that the plasma marker is introduced into the circulation and that a percentage of the total plasma volume is filtered per unit time. The model was used to examine issues related to plasma marker introduction and to departures from equilibrium during a measurement period.

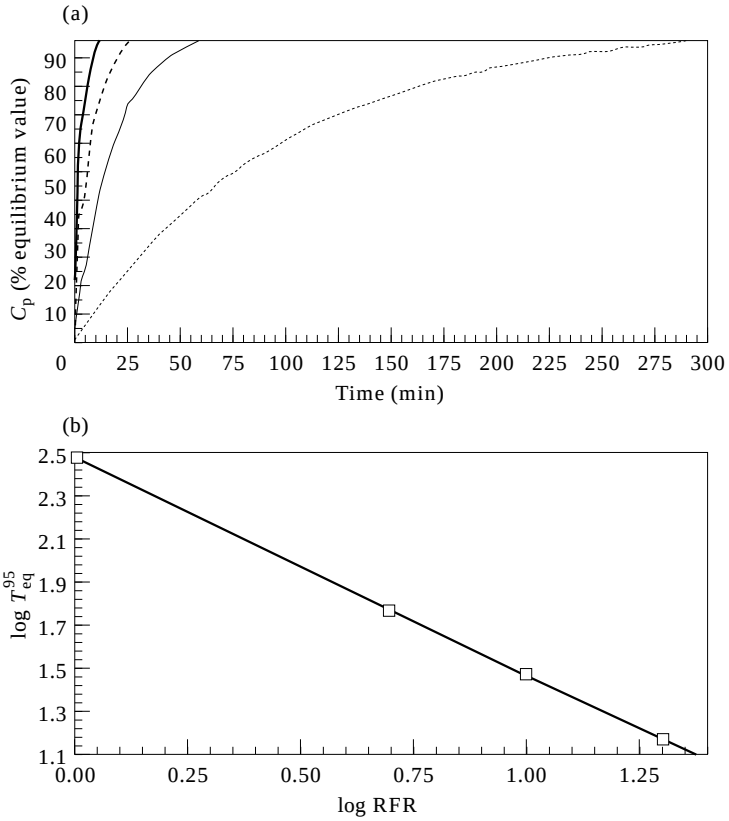

FIG. 1. (a) Plasma marker concentration (% equilibrium value) is plotted as a function of time (min). C_p approaches 95% of the equilibrium value at different rates, depending on RFR. The shape of the curves is the same; the effect of RFR is to either stretch or compress the curve on an absolute time scale. (b) Plotting log T_{eq}^{95} as a function of log RFR yields a straight line described by the general relationship: log $T_{eq}^{95} = 2.47 - (\log \text{RFR})$, or $T_{eq}^{95} = 295/\text{RFR}$. This relationship may be important during experimental design. RFR (%min^{-1}): (– – –) 1%, (——) 5%, (----) 10%, (——) 20%.

5. Introduction of a Filtration Marker

There are two important questions related to the introduction of the filtration marker. First is the length of time required after the initiation of marker introduction before the plasma concentration will begin to reach stability. Is there a relationship between the marker introduction rate and the time to reach a stable plasma concentration? Second is the amount of filtration marker that must be introduced in order to obtain a measurable plasma marker concentration. Enough marker must be introduced so that a measurable plasma concentration is obtained. However, using more marker than the amount required is not only wasteful, but could be harmful to the experimental animal.

The time needed for filtration marker concentration to reach stability in the plasma is related to the amount of time needed to reach equilibrium (T_{eq}) in the system. That is, marker concentration will become more stable as $\mathring{V}_{ex}$ approaches $\mathring{V}_i$. The time required for the system to reach equilibrium is inversely related to the RFR. Figure 1(a) shows the relationship between the time needed to reach equilibrium and RFR. The general shape of the plasma marker concentration curve through time is the same at any RFR. The initial concentration increases quickly as compared with the asymptotic approach to the equilibrium concentration. The effect of RFR on the concentration curve is to either stretch or compress the curve on an absolute time scale.

The relationship between T_{eq} and RFR can be described mathematically after plotting T_{eq} as a function of RFR. Since the plasma marker concentration curve approaches equilibrium asymptotically, T_{eq}^{95} may be defined as the plasma marker concentration at which 95% of the expected equilibrium is reached. The relationship between T_{eq}^{95} and RFR is shown in Fig. 1(b). The equation describing this relationship is:

$$T_{eq}^{95}\,(\text{min}) = 295/RFR(\%\ \text{min}^{-1}) \qquad (7)$$

Note that neither introduction rate nor the absolute amount of marker added into the circulation influence the T_{eq}^{95}. Instead, RFR is the fundamental determinant

of this parameter. T_{eq}^{95} is determined by the experimental animal and cannot be altered by the measurement technique employed. The relationship between RFR and T_{eq}^{95} is an inverse one. Therefore, large animals will require a longer period of time to reach equilibrium than small animals and ectothermic animals will require a much longer time period to reach equilibrium than endothermic animals.

The marker introduction rate needed for a measurable plasma concentration is influenced by three parameters. These are the RFR, P_v, and the total amount of plasma available for sampling. The plasma marker concentration can be expressed as R_{total} divided by P_v:

$$C_p(\text{units ml}^{-1}) = R_{total}(\text{units})/P_v(\text{ml}) \quad (8)$$

At equilibrium, the marker excretion rate and introduction rate are equal, so eqn (4) becomes:

$$R_{total}(\text{units}) = \dot{V}_i(\text{units min}^{-1})/RFR(\%\ \text{min}^{-1}) \quad (9)$$

Substituting this expression for R_{total} in eqn (8) shows that the plasma marker concentration at equilibrium is equal to:

$$C_p(\text{units ml}^{-1}) = [\dot{V}_i(\text{units min}^{-1})/ RFR(\%\ \text{min}^{-1})]/P_v(\text{ml}). \quad (10)$$

This equation reveals that the C_p is a direct function of the introduction rate. While RFR and plasma volume also determine plasma concentration, only introduction rate is under the control of the investigator.

As a practical example, osmotic minipumps have been successfully used as marker delivery devices in avian GFR measurements (Goldstein & Braun, 1988; Roberts & Dantzler, 1989; Rothschild & Goldstein, 1990; Williams *et al*., 1991; Goldstein &Rothschild, 1993). These studies generally cite the volume capacity and pumping rate, the amount or concentration of marker loaded in the pumps, and the time period between minipump implantation and GFR measurements. These papers report GFR measurements made on birds ranging from about 18 to 158 g in body mass. Suppose a comparative physiologist wanted to follow these protocols for GFR measurements in a comparably sized lizard (100 g). How should the experimental specifications be adjusted for this animal? The RFR of birds in this size range is expected to be from about 8.5 to 17% min^{-1}. The lizard is expected to have an RFR of about 0.19% min^{-1}. From eqn (10), the lizard will have a C_p that is about 45 to 90 times higher than the birds assuming everything else is equal. Thus, the $\dot{V}_i$ could be greatly reduced by lowering the concentration of loaded marker, resulting in the savings of an expensive marker. From eqn (7), the T_{eq}^{95} in the lizard will also be 45 to 90 times longer than in the comparable birds. There may be need to wait longer before measurements are taken.

6. Departures from Equilibrium

Any change in GFR, marker introduction rate, or plasma volume will create a departure from stability in excretion rate and plasma marker concentration. Modulation of GFR is an important point of osmoregulation in non-mammalian vertebrates (Braun & Dantzler, 1972; Calder & Braun, 1983; Yokota *et al*., 1985; Dantzler, 1988; Goldstein & Rothschild, 1993); taking plasma samples for marker concentration will change the plasma volume; and marker introduction rate may not be perfectly constant.

The rate of change that occurs in the variables used to estimate GFR during a non-equilibrium state is inversely proportional to RFR. This relationship means that there are practical limitations to the measurement of plasma marker concentration and marker excretion rate which are related to RFR; and that different animals have different inherent variability in marker concentration and excretion rate depending on their RFR. Two examples illustrate these trends. The first of these uses the single injection method for measuring GFR and the second uses a hypothetical GFR change.

The single injection method does not use a continuous introduction of filtration marker as in standard clearance techniques. Instead, a single bolus of marker is introduced directly into the circulation. Because the filtration marker introduction rate is zero, the marker will immediately begin to disappear from the plasma. The higher the RFR, the more quickly the rate of marker disappearance. The ratio between the amount of marker introduced and the area under the plasma marker disappearance curve is used to estimate GFR (Mitch & Walser, 1986).

Figure 2 shows the relationship between RFR and plasma marker disappearance curves. Plotting the time needed to reach 5% of the initial plasma marker concentration shows that this time is the same needed to reach 95% T_{eq}. That is, the time needed to reach the 5% level ($T_{5\%}$) is described by the same relationship as shown in eqn (7):

$$T_{5\%}(\text{min}) = 295/RFR(\%\ \text{min}^{-1}). \quad (11)$$

An animal with an RFR of 1% will reduce plasma marker concentration to 5% of the original concentration in 300 min. In contrast, an animal with

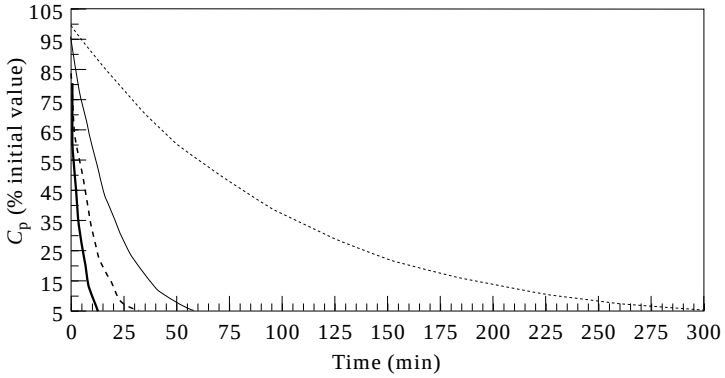

FIG. 2. Plasma marker concentration (% initial value) is plotted as a function of time (min) as in a single injection measurement of GFR. Since marker introduction rate is zero, the marker begins to leave the plasma at the start of the experiment. The rate at which the marker concentration approaches 5% of the initial value is dependent on RFR: the higher the RFR, the higher the rate of marker disappearance. RFR (%min^{-1}): (– – –) 1%, (——) 5%, (----) 10%, (——) 20%.

an RFR of 20% will reach the same level in less than 15 min.

As in single injection measurements, any changes in filtration marker concentration will occur at a rate inversely proportional to RFR. Figure 3(a) shows a hypothetical 18% reduction in equilibrium GFR and back again over a 40 min time period. Figure 3(b) shows how plasma levels (% of equilibrium value) change in response to this change in GFR. An animal with a higher RFR will have a marker concentration that is inherently more labile on an absolute time-scale than an animal with a lower RFR. Transient

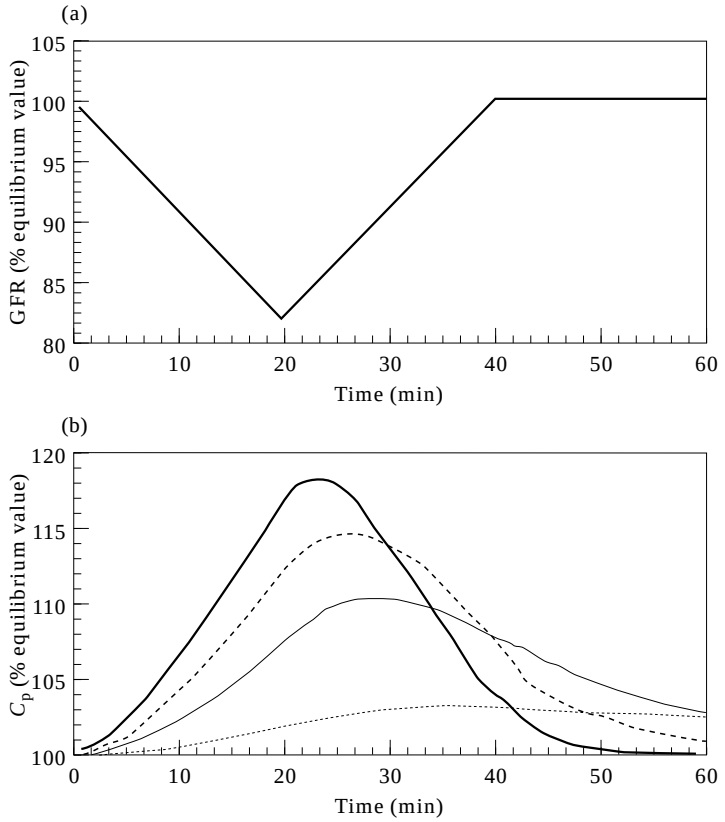

FIG. 3. (a) GFR (% equilibrium value) is plotted as a function of time (min). This hypothetical example shows a reduction to 18% of equilibrium GFR and back again in a 40 minute period. (b) Plasma marker concentration (% equilibrium value) plotted as a function of time (min) changes as a result of the GFR change in (a). The same relative change in GFR leads to disproportionate relative changes in plasma marker concentration. Notice that the changes in plasma marker concentration more closely follow the GFR change when RFR is high. RFR (%min^{-1}): (– – –) 1%, (——) 5%, (----) 10%, (——) 20%.

increases or decreases in GFR may be especially problematic. Such temporary changes in GFR can cause dramatic changes in plasma marker concentration which quickly return to their previous levels. Concomitant changes in marker excretion rate may be missed, since excretion rate is measured over a time continuum.

The avian GFR protocols using minipumps (Goldstein & Braun, 1988; Roberts & Dantzler, 1989; Rothschild & Goldstein, 1990; Williams *et al*., 1991; Goldstein & Rothschild, 1993) can exemplify the potential for measurement error. In the laboratory, one method for estimating excretion rate is by collecting total excreted marker over 3 or more hours (Goldstein & Braun, 1988; Roberts & Dantzler, 1989; Williams *et al*., 1991; Goldstein & Rothschild, 1993). In field studies (Rothschild & Goldstein, 1990; Goldstein & Rothschild, 1993) excretion rate is assumed to be equal to manufacturer's specified pump rate, given that equilibrium has been reached. In all cases, GFR measurements are based on either a mean excretion rate or an estimated excretion rate. In contrast, blood samples are taken at a single point in time at the end of the experimental period. Since modulation of GFR is an important point of regulation in some animals, it is likely that the stress of blood sampling affects GFR. When this occurs, the calculated GFR does not represent the experimental GFR, but largely reflects the effects of handling. Again notice that the departures from equilibrium are strongly influenced by an animal's RFR (Fig. 3). At least one study (Rothschild & Goldstein, 1990) dealt with this problem by taking blood samples within two minutes of recapture.

7. Conclusion

Differences in physiological time between animals of distinct phylogeny and body mass are intimately related to the practical measurement of GFR. This paper has been an attempt to discuss some of the most basic elements of any GFR measurement. Even at this most basic level, some trends may seem unexpected or even counter-intuitive, such as the fact that T_{eq} is determined by RFR and not by the rate of marker introduction.

It is important to tailor the appropriate measurement protocol to the experimental animal. The protocol used to measure GFR in a reptile would be of little use to an investigator attempting to measure GFR in a small bird or mammal. The examination of quantitative relationships can serve as a conceptual framework for planning purposes. While examination of these relationships does not replace experimentation, examination of general relationships can complement experimentation. What is presented here is but an example of the need to consider physiological timescale when planning physiological measurements. There are almost certainly a number of other experimental procedures greatly influenced by differences in physiological time and this influence should be carefully considered when initiating measurements.

I am very grateful to all of those who took time to review this paper and make critical suggestions.

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