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# Plasma volume changes as a result of equilibrium dialysis

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Equilibrium dialysis techniques are extensively used to determine the extent of drug-plasma protein binding interactions. It has now been recognized that corrections have to be made for the loss of drug from the plasma to the buffer during dialysis (Behm & Wagner 1979). Conventionally, free fractions have been calculated in dialysis systems as the ratio of the drug concentration in the buffer to that in the plasma. Since the binding parameters obtained from these experiments are protein concentration-dependent, it is implicit in the above approach that no change in plasma protein concentration occurs during the dialysis process. A body of evidence exists to suggest that this assumption is not valid (Kurz et al 1977; Abel et al 1979) and certain groups of workers have taken steps to counteract osmotic water shifts that result in the dilution of the plasma protein concentrations (Kurz et al 1977; Mueller & Potter 1980). Considering the importance of possible volume shifts, it seems amazing that few literature values of the magnitude of this change exist (Abel et al 1979). In this communication we report the direct measurement of these volume changes and show how correction for these changes can affect mass balance recovery data.

The dialysis system used has been reported in detail elsewhere (Lockwood et al 1982). One ml volumes of plasma, obtained from 15 young healthy male subjects, were subjected to 8 h dialysis against a 0·093 M pH 7·4 phosphate buffer (0·696 g $KH_2PO_4$, 0·138 $NaH_2PO_4 \cdot H_2O$, 2·25 g NaCl, made up to 500 ml with $H_2O$) at 37 °C. The buffer volume at the start of dialysis was 3 ml. At the end of the dialysis period, dialysed plasma, un-dialysed plasma from the same subjects and dialysed buffer were diluted as necessary and their protein concentrations were assayed (Lowry et al 1951). The results of the plasma protein assays are presented in Table 1. It can be seen that there is an appreciable, statistically significant decrease in plasma protein concentration following dialysis. Since the protein concentrations measured in the buffer compartment accounted for less than 0·1% of the initial plasma protein (0·077% ± 0·05%), the reductions observed are not accountable in terms of protein leakage. It is therefore proposed that the decrease in plasma protein concentrations observed was due to an osmotic movement of fluid into the plasma compartment (as a consequence of it containing non-diffusable protein molecules). It should be noted that the mean percent *decrease* in plasma protein concentration (16·5%) corresponds to a 19·7% *increase* in the volume of the plasma compartment.

A consequence of this volume change effect is that it can be difficult to obtain mass balance data from dialysis systems. If a volume shift does occur and it is ignored, the direction of this shift could lead to an underestimation of the total drug recovery. Table 2 illustrates this point. Five dialysis studies were carried out using one subject's plasma. The plasma samples were spiked with unlabelled and radiolabelled ibuprofen over a range of concentrations (1·3–130 μg $ml^{-1}$). The initial activity of the radiolabel in the plasma ($DPM_I$) was known. At dialysis equilibrium the activity per unit volume ($DPM_E$ $ml^{-1}$) in plasma and buffer was measured. The magnitude of any volume shifts was measured by assaying protein concentrations before and after dialysis as already described. The percent initial activity present in plasma and buffer was then calculated: (a) assuming no volume shift, and (b) assuming a volume shift of the magnitude estimated by the protein analysis using equation 1:

$$\% \text{ initial activity} = \frac{DPM_E/ml \times V}{DPM_I} \times 100 \qquad (1)$$

when V represents the volume of the compartment under consideration and $DPM_E/ml$ represents the measured activity in that compartment.

As can be seen from Table 2, using the uncorrected volumes in equation 1 can lead to a gross underestimation of the total recovery. Under these circumstances the 'loss' of drug could falsely be attributed to non-specific binding to the dialysis membrane or other components of the dialysis system.

This report has shown that a volume shift, causing an increase in plasma volume, occurs under the equilibrium dialysis conditions described. The magnitude is similar to that reported by Abel et al (1977). This result

Table 1. A summary of the pre- and post-dialysis plasma protein concentrations.

| | Plasma protein concentration (mg $ml^{-1}$) | | |
|---|---|---|---|
| | Before dialysis | After dialysis | % Decrease |
| Mean | 67·4 | 55·8* | 16·5 |
| Range | 82·3–53·7 | 64·2–45·5 | 25·1–4·11 |
| s.d. | 9·6 | 5·2 | 6·2 |
| c.v. (%) | 14·2 | 9·3 | 37·6 |

* Statistically different at $P < 0·001$ by paired $t$-test.

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Table 2. Effects of ignoring volume change when trying to calculate mass balance from dialysis studies.

| Run no | Initial Ibuprofen concn (μg ml$^{-1}$) | Percent of initial activity recovered: Protein compartment no change | Protein compartment volume change | Buffer compartment no change | Buffer compartment volume change | Total recovery no change | Total recovery volume change |
|---|---|---|---|---|---|---|---|
| 1 | 1·3 | 84·7 | 97·0 | 1·6 | 1·4 | 86·3 | 98·4 |
| 2 | 10 | 84·5 | 96·9 | 1·6 | 1·5 | 86·1 | 98·4 |
| 3 | 50 | 87·5 | 96·6 | 2·1 | 2·0 | 89·6 | 98·6 |
| 4 | 100 | 80·5 | 93·4 | 3·0 | 2·9 | 83·5 | 96·3 |
| 5 | 130 | 75·8 | 92·5 | 3·3 | 3·1 | 79·1 | 95·6 |

is important since it implies that protein binding values calculated conventionally have all been estimated at significantly reduced plasma protein concentrations even when the volume shift is recognized (Abel et al 1977). The novel approach towards estimating drug concentrations in the plasma at dialysis equilibrium recently reported by Giacomini et al (1982) does nothing to overcome this problem. As pointed out in that paper the assumption of complete drug recovery is often difficult to validate. An approach as outlined above might prove useful in this respect.

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# Diurnal variation in the phlogogenic response of rats to inflammatory agents

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It is well known that the responses in man and animals to various environmental stimuli and drugs vary diurnally (Reinberg 1967; Halberg 1969; Walker et al 1981). Such diurnal variation includes the response to histamine (DeVries et al 1962) and allergens (Reinberg et al 1965). The incidence of asthma appears to follow a certain diurnal pattern (Reinberg et al 1963). It has also been shown that the inflammatory response of the human skin to house dust and penicillin exhibits circadian rhythmicity (Reinberg et al 1969). More recently, Labrecque et al (1981) indicated that carrageenan-paw oedema formation in the rat follows circadian pattern. The purpose of the present study was to determine the diurnal response to two inflammatory agents, croton oil and polymyxin B.

*Methods*

In this experiment, adult male Sprague-Dawley rats, 120–150 g, were adapted for a minimum period of three weeks to a temperature of 21 ± 1 °C in an environmental chamber equipped to provide 23·25 cd m$^{-2}$ of cool white fluorescent light. The chamber was equipped with an automatically timed 12 h dark-12 h light period which lasted from 0800 to 2000 h daily. Standard pellet diet (Purina, St. Louis, MO) and water were freely available. The phlogogenic response to croton oil was assessed by the method described by Glenn et al (1978). In this method, 0·5 ml of 5% croton oil containing 0·1% $H_2SO_4$ was applied to the right ear of the animal while the left ear was left as control. Four hours later the rats were killed and each ear was excised and weighed. The difference between the weights of the treated and control ears was taken as a measure of oedema formation in response to croton oil. In assessing the phlogogenic effect of polymyxin B, the method of Bertelli & Soldani (1979) was used. One tenth of a ml of 1 μg ml$^{-1}$ polymyxin B in sterile 0·9% NaCl solution was injected s.c. into the right hand paw and 0·1 ml of sterile 0·9% NaCl was injected into the left hind paw. Paw volumes were measured 1 h later by mercury plethymography according to a method described by Winter et al (1962). The difference in paw volumes was taken as a measure of the phlogogenic effect of polymyxin B. The inflammatory response to each agent was determined in separate groups of 8 rats every 4 h over a 24 h period. The application or the administration of the phlogogenic agent during the dark period was performed with minimum disturbance to the animals using dim light (0·16 cd m$^{-2}$) in the chamber. The statistical significance of data obtained was assessed by analysis of variance test (Steel & Torrie 1960).

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