APPENDIX 1 - DETAILED MODELING METHODS AND EQUATIONS OF THE MODELS (TO BE PUBLISHED ON THE ANESTHESIOLOGY WEBSITE ONLY)

The symbols used in the model equations are common between models where possible. Each is been defined only when first introduced.

SIMPLE TIME DOMAIN MODELS

The time domain models were those in which the independent variable was time (t). In defining these models, it is important to distinguish between the independent variable, the dependent variables of the models, and the parameters of the models. By way of example, the equation of a single compartment flow-limited model will be examined in detail, as this model requires only a single differential equation. It is commonly used in physiological pharmacokinetic models, and is the most basic description of the kinetics of a lipophilic or highly diffusable drug in an organ.

Single flow-limited compartment model

$$V_b . \frac{dC_{sag}}{dt} = Q_b . (C_{art} - C_{sag}) \quad ...(2)$$

The dependent variables of this model were those that changed with time: C_{art} and C_{sag}, which represent the drug concentrations in arterial and sagittal sinus blood respectively, and Q_b the cerebral blood flow. The parameter V_b is the apparent distribution volume of the brain and is independent of time. For this model, V_b is the product of the real volume of the brain and the brain : blood partition coefficient.

The direction of blood flow in the model (arterial to venous) dictates that the dependent variables can be classified as either "inputs" or "outputs" to the model. While it is possible to experimentally manipulate and measure C_{art} and Q_b (the "inputs") and observe the resultant C_{sag} (the output), the converse is clearly impossible. The former was the experimental paradigm used in this paper. Given the time-courses of the input variables, the question asked was whether there was a parameter value for the model (V_b) that agreed with the observed time-course of output concentrations (C_{sag}). In modeling terms, this required finding a solution for C_{sag} in Eqn 2, given values of C_{art}, Q_b and V_b.

As Eqn 2 is a differential equation, it can only be solved analytically in special circumstances. For example, if C_{art}, is a fixed at a value of 2 the equation becomes.

$$\frac{dC_{sag}}{dt} = \frac{Q_b}{V_b}.(2 - C_{sag}) \quad ...(3)$$

Standard calculus methods can be applied to this equation; to give the solution:

$$C_{sag} = 2(1 - \exp^{-\frac{Q_b}{V_b}t}) \quad ...(4)$$

In this case, the sagittal sinus concentrations will rise in an exponential manner until they are equal to the value of the arterial concentrations (2). The rate constant of the rise will be dictated by Q_b/V_b That is, they will rise quicker to meet the arterial when the blood flow is high, or when the volume of distribution in the brain is low.

Empirical forcing functions

For the in vivo experiments the same principles apply, but it was clearly impossible for C_{art} and Q_b to be constrained to constant values. The former rose and fell in a multi-exponential manner during and after the drug infusion, while the latter changed from its baseline value presumably due to drugs effects and other experimental influences. Although these changes were measured, their incorporation into Eqn. 2 makes the equation difficult, if not impossible, to solve using the analytical method discussed above. However, it can be solved using numerical methods via a differential equation solver computer program. For the present paper, "Scientist for Windows" was used which utilises the Episode numerical integration routine. At the heart of this method is the integration of the equation by calculating the "area under curve" of extremely small time-intervals. Consequently, the model cannot be solved numerically using the discrete measured data points for C_{art} and Q_b, but must use a continuous function so that values of C_{art} and Q_b at any time can be determined. The data were therefore fitted to empirical mathematical functions to interpolate the values between the measured data - its form is not important other than it fulfills this requirement. The resultant empirical "forcing functions" were then incorporated into the models, and were used to "force" the dependent variable to follow the time-course of the observed data. In this example, C_{art} was fitted to a biexponential equation

and Q_b was fitted to a polynomial. If they were represented by functions f_{art} and f_b, respectively, then the system of equations to be solved becomes.

$$\begin{aligned} C_{art} &= f_{art}(t) \\ Q_b &= f_b(t) \\ V_b . \frac{dC_{sag}}{dt} &= Q_b . (C_{art} - C_{sag}) \end{aligned} \qquad \text{...(5)}$$

This process of representing some aspects of the model as forcing functions is sometimes known as hybrid modeling. In summary, it accounts for the influence of the known dependent variables on the time-course on the observed output C_{sag} concentrations.

During the curve-fitting process, various values of V_b were tried in order to minimise the difference between the observed and predicted values of C_{sag}. Again, Scientist for Windows was used which incorporates the Powell variant of the Levenberg-Marquardt curve-fitting routine. Good agreement between the observed and predicted sagittal sinus concentrations were taken to mean that the structure of the model and the chosen value of V_b were able to describe the experimental system, but not that a given model was correct. However, models that fit the data poorly were able to be excluded as faithful descriptions of the experimental system.

Simultaneous modeling of two blood flow states

A feature of the present paper was that the kinetics of thiopental and propofol were measured at both low and high states of cerebral blood flow. The time-course of the dependent variables arterial concentration and cerebral blood flow were measured at each flow state and each were each fitted to empirical forcing functions as described previously. These will represented as $C_{art,lo}$ and $Q_{b,lo}$ and $C_{art,hi}$ and $Q_{b,hi}$, respectively. The question asked was whether there was a model with a single set of parameters that could account for the observed time-courses of the sagittal sinus concentrations at low and high blood flows for each flow state ($C_{sag,lo}$ and $C_{sag,hi}$, respectively). This was achieved by simultaneously fitting the two models (one for the low flow state, one for the high flow state) that had common parameter values. An overview of the process involved is shown in Figure 7.

The system of equations for a single flow-limited compartment model were as follows.

$$
\begin{aligned}
C_{art,lo} &= f_{art,lo}(t) \\
C_{art,hi} &= f_{art,hi}(t) \\
Q_{b,lo} &= f_{b,lo}(t) \\
Q_{b,hi} &= f_{b,hi}(t) \\
V_b \cdot \frac{dC_{sag,lo}}{dt} &= Q_{b,lo} \cdot (C_{art,lo} - C_{sag,lo}) \\
V_b \cdot \frac{dC_{sag,hi}}{dt} &= Q_{b,hi} \cdot (C_{art,hi} - C_{sag,hi})
\end{aligned}
\qquad \ldots(6)
$$

Note that the parameter V_b is the same parameter for each differential equation. This was the general modeling method used in the paper, but various other structural models were substituted for the single flow-limited compartment model to examine their ability to account for the observed data. The models examined are listed below, with a brief rationale for their inclusion. A schematic representation of each model is shown in Table 1.

Traditional membrane-limited model

The membrane limited model is used to describe drugs that are not sufficiently lipophilic to be described using a single flow-limited compartment model. A diffusion barrier was added, dividing the organ into two well-stirred compartments. In addition to the symbols used above, C_{deep} was the concentration in the "deep" or non-vascular compartment of the model, V_{deep} was its apparent volume and PS_{deep} was the inter-compartmental clearance describing membrane permeability (the product of permeability and surface area).

$$
\begin{aligned}
V_b \cdot \frac{dC_{sag}}{dt} &= Q_b \cdot (C_{art} - C_{sag}) + PS_{deep} \cdot (C_{deep} - C_{sag}) \\
V_{deep} \cdot \frac{dC_{deep}}{dt} &= PS_{deep} \cdot (C_{sag} - C_{deep})
\end{aligned}
\qquad \ldots(7)
$$

By not fixing the value of the V_b, no assumption was made about the physical location of the diffusion barrier within the organ. At a microscopic level, it may represent the endothelium, the cell membrane or even the membrane surrounding intra-cellular structures. At a macroscopic level, it may represent an area of poorly perfused tissue within or surrounding an organ.

TIME DOMAIN MODELS INCORPORATING DISPERSION

The concept of dispersion is best illustrated by considering the output (venous concentrations) from a model of an organ if drug enters the organ as a short impulse. This is often represented mathematically as a square-wave arterial concentration peak of infinite height and infinitely short duration (Dirac function). Conceptually, if can be thought of as a population of drug molecules entering the organ simultaneously. In practice, a situation approximating impulse administration can be achieved using isolated perfused organ preparations in a non-recirculating system if drug is injected as a rapid bolus into the afferent (arterial) perfusate. Drug concentration is measured continuously in the efferent (venous) perfusate over the next few minutes. Typically, for drugs the efferent concentration profile (sometimes called the impulse-response function) will start at zero at the time of the injection, then after a short lag rise to a maximum value before declining back to zero. An example concentration time-curve is shown in Figure 8. Studies of intravascular markers (i.e. confined within the blood vessels during transit through an organ) show a similar shape to that described above. This suggests that one important factor contributing to the shape of this curve for drugs (although modified by their distribution volume) is the different lengths of the capillary pathways through the organ, and the dispersion of the input peak by mixing at vascular junctions etc.

Both the single flow-limited compartment model and the traditional membrane limited model do not account for drug dispersion within an organ. For both models, following impulse drug administration, drug molecules are assumed to diffuse instantaneously throughout the vascular compartments and to begin leaving the organ via venous blood immediately after the injection. The maximum venous concentration therefore occurs at the time of the injection, after which the venous concentrations decline either exponentially (single flow-limited compartment model) or bi-exponentially (traditional membrane limited model) as shown in Figure 8. This behaviour is rarely seen experimentally, and it is generally accepted that these simple models are poor descriptions of impulse-response studies. However, it is less clear to what extent accounting for dispersion is necessary for studies of organ drug kinetics in vivo where input drug concentrations change more slowly that those of impulse studies. The intention in examining the following models was to examine the contribution of this intra-organ dispersion in vivo. There are a variety of organ models that can produce impulse-response functions

with the required general shape, but with relatively subtle differences between models. The following models were examined for this purpose.

2 tanks in series model

Linking two or more single-flow limited compartments ("tanks") in series is a simple way of adding an element of dispersion to a organ model. Models with more than two tanks were not used after initial trials showed adding additional tanks produced imprecise parameter estimates during curve-fitting. In addition to the symbols used above, C_1 is the concentration in the "upstream" compartment of the model, V_1 is its apparent volume.

$$\begin{aligned} V_1.\frac{dC_1}{dt} &= Q_b.(C_{art} - C_1) \\ V_b.\frac{dC_{sag}}{dt} &= Q_b.(C_1 - C_{sag}) \end{aligned} \quad ...(8)$$

Compilation model

This model adds both "deep" and "upstream" compartments to a single flow-limited compartment model. It is therefore a compilation of the "2 tank in series" and "membrane-limited" model, as reported previously[16].

$$\begin{aligned} V_1.\frac{dC_1}{dt} &= Q_b.(C_{art} - C_1) \\ V_b.\frac{dC_{sag}}{dt} &= Q_b.(C_1 - C_{sag}) + PS_{deep}.(C_{deep} - C_{sag}) \\ V_{deep}.\frac{dC_{deep}}{dt} &= PS_{deep}.(C_{sag} - C_{deep}) \end{aligned} \quad ...(9)$$

LAPLACE DOMAIN MODELS INCORPORATING DISPERSION

One method of incorporating an element of dispersion in an model, as used by Roberts and co-workers[17,18], has been the use of a statistical distribution curve to describe the transit times of drug through the organ. The properties of the dispersion process are summarized using terms describing

the properties of the transit-time distribution curve. These types of dispersion models can only be solved practically by transforming the model into the Laplace domain rather than the more conventional use of the time domain. This a relatively complex manipulation of differential equations that is standard practice in many fields (engineering in particular). The advantage of equations transformed into the Laplace domain is that the process of convolution (e.g. of an arterial input function and a transit time distribution curve) is reduced to an operation of multiplication, while the converse process of deconvolution becomes division. The final equation of the model must be transformed back into the time-domain, which can be done numerically. Again, Scientist for Windows was used for this process, which makes use of Weeks' method for the inversion.

Single compartment dispersion model

The Laplace transform of the venous effluent drug concentration was found from the product of the Laplace transform of the arterial drug concentration and the Laplace transform of an inverse Gaussian transfer function[17]. The coefficient of variation was taken as twice the quotient of the time constants of perfusion (MTT) and axial diffusion (τ_{Dax})[18]. An overbar on a variable indicates a Laplace transform, and s is the Laplace variable.

$$MTT = \frac{V_b}{Q_b} \quad ...(10)$$

$$CV^2 = \frac{2.MTT}{\tau_{Dax}} \quad ...(11)$$

$$\overline{C}_{sag} = \overline{C}_{art}.\exp\left(\frac{1-\sqrt{1+2.CV^2.s.MTT}}{CV^2}\right) \quad ...(12)$$

Two compartment dispersion model

In this model, the transit time of a drug molecule were given by the vascular transit time and the tissue residence time[19]. The overall tissue residence time was determined by the number of excursions into the tissue, described as a Poisson process, and the conditional probability density function of the residence time given the number of excursions. This is given by the following replacements in the single compartment dispersion model. The Laplace variable s was replaced with:

$$s + K - \frac{K}{1 + \frac{v}{K.\tau_{Dlat}}.\sqrt{\tau_{Dlat}.s}.\tanh\sqrt{\tau_{Dlat}.s}} \quad ...(13)$$

$$\text{where } K = \frac{PS}{(0.04).Vb} \quad ...(14)$$

and MTT with:

$$MTT_{cap} = \frac{(0.04).V_b}{Q_b} \quad ...(15)$$

A new expression for CV^2 was used that contained terms describing the contribution of vascular dispersion, permeation and lateral diffusion in the tissue

$$CV^2 = \frac{2.MTT_{cap}}{\tau_{Dax}} + \frac{Qb.2.v^2}{PS.(1+v)^2} + \frac{2.\tau_{Dlat}.v}{3.MTT_{cap}.(1+v^2)} \quad ...(16)$$

where v=25 and was the quotient of tissue and capillary blood apparent volumes, and τ_{Dax} and τ_{Dlat} were the axial and lateral diffusion time constants, the latter fixed at L^2/D. L was the maximum lateral diffusion distance set at 0.002 cm; D was the drug diffusivity at 37^{o}C in the deep compartment approximated as $2.46*10^{-7}$ cm^2/sec for both drugs; and PS was the same as for the membrane-limited model, with the diffusion barrier assumed to be at the blood-tissue interface.

Reference model for the Laplace domain - Membrane limited model

This model was used as a common reference model between the time and Laplace domains, as they differed in the method used to account for time-dependent changes in cerebral blood flow from baseline values. This also provide a check that the two methods of solving models were comparable.

$$k_1 = \frac{Q_b}{V_b}$$

$$k_2 = \frac{PS_{deep}}{V_{deep}} \quad ...(17)$$

$$\overline{C}_{sag} = \frac{\overline{C}_{art}.k_1}{(s + k_1 + k_2) - \frac{{k_2}^2}{(s + k_2)}}$$