# Plasma Concentration Clamping in the Rat Using a Computer-Controlled Infusion Pump

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We have developed a computer-controlled infusion pump to achieve rapidly and then maintain stable plasma thiopental concentrations in rats. Initially we derived the parameters of a triexponential pharmacokinetic model for thiopental, administered as a brief infusion to 10 rats, using nonlinear regression and standard pharmacokinetic equations. These parameters were incorporated into the pharmacokinetic model of a computer-controlled infusion pump. In a second group of animals this device was used to maintain three consecutive target thiopental concentrations ranging from 5 to 100 μg/ml in a stepwise fashion. Arterial blood gases were kept normal through controlled ventilation when necessary. The plasma thiopental concentrations in this second group of animals were generally higher than the target concentrations. The bias in pump performance (median prediction error) was +25%, and the inaccuracy (median absolute prediction error) was 26%. We fit the parameters of a three-compartment model to the plasma thiopental concentrations observed in the second group of animals. This produced a second set of thiopental pharmacokinetic parameters with the unique characteristic of having been derived from a computer controlled infusion study. These parameters were tested prospectively with a computer-controlled infusion pump in a third group of animals. This second set of thiopental pharmacokinetic parameters performed better, with a median prediction error of 0% and a median absolute prediction error of 15%. This study shows that it is possible to achieve rapidly and maintain steady plasma thiopental concentrations in the rat. Our results suggest that it is feasible to derive robust pharmacokinetic parameters from unusual drug dosing approaches, such as employed by a computer-controlled infusion pump. The ability rapidly to clamp plasma drug concentrations at desired targets in small laboratory animals will facilitate research into the relationship of plasma and tissue concentration to drug effect.

KEY WORDS: computers; infusion pumps; infusions, intravenous; pharmacokinetics; thiopental; rats; computer-controlled drug delivery; nonlinear regression.

## INTRODUCTION

Computer-controlled infusion pumps (CCIPs) have been developed for clinical use in patients to achieve rapidly and maintain stable plasma concentrations of lidocaine (1), fentanyl (2–4), alfentanil (5,6), thiopental (7), and propofol (8). These devices are based on algorithms originally proposed by Krüger-Theimer (9) and implemented by Schwilden (10). The algorithms involve solving the standard polyexponential (i.e., multicompartmental) disposition function of intravenous drugs for the infusion rate necessary to maintain a desired target drug concentration. This approach has been explained in detail previously (11). Investigators in the above-referenced studies (1–8) have reported satisfactory performance with CCIPs in humans. However, these devices have not previously been scaled down for research in small laboratory animals.

To facilitate our research in thiopental pharmacodynamics, the goal of the present investigation was to develop a CCIP that allows us accurately and reproducibly to "clamp" the thiopental concentration in an instrumented rat at any desired concentration within the therapeutic range. We initially performed a standard pharmacokinetic analysis in rats by measuring plasma thiopental concentrations following a brief thiopental infusion. These initial pharmacokinetic parameters were used in a CCIP to administer thiopental to a second set of rats. Several systematic errors were observed between the measured and the target thiopental concentrations. We then derived a second set of thiopental pharmacokinetic parameters from our initial CCIP results. This second set of thiopental parameters was prospectively tested using a CCIP in a third set of rats.

Our system allows us accurately and reproducibly to clamp the plasma thiopental concentration anywhere within the therapeutic range. Once the plasma concentration has been clamped at a pseudo-steady-state concentration, all body tissues will equilibrate with the plasma concentration after a suitable period of time. This system permits direct analysis of plasma concentration–drug effect relationships without the confounding variable of changing plasma and tissue drug concentrations, such as occurs in studies using a bolus or zero-order infusion.

## METHODS

### Animal Model

With the approval of our Animal Care Committee, we studied 28 male Wistar rats (300–400 g, Harlan-Sprague-Dawley, Indianapolis, IN) over three separate studies. Two days prior to each study catheters were placed in the jugular vein and in the intraabdominal aorta via the caudal tail artery of each animal under isoflurane anesthesia. The catheters were kept patent with heparin solution. The animal model has been described in detail previously (12). During studies 2 and 3 (described below) the animals were mechanically ventilated when the target plasma thiopental concentration exceeded 40 μg/ml because our previous work had demonstrated significant respiratory depression at thiopental concentrations above 40 μg/ml. During each study, arterial blood gases were checked periodically to assure normal pH and adequate oxygenation and ventilation. The animals received normal saline at 2 ml/hr to compensate for urine and insensible fluid losses. Following each blood sample the animals also received a bolus of normal saline that was 2.5

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times the volume of the blood sample. The total phlebotomy over the course of the study was a maximum 3 ml, which was replaced by intravenous saline. The animals were maintained on a heating pad whose temperature was varied as necessary to maintain normothermia.

### Computer-Controlled Infusion Pump

Our CCIP was a Toshiba T-3100 computer running MS-DOS, connected to a Harvard Apparatus Pump 22 (Harvard Apparatus, South Natick, MA), with a serial interface. The algorithms incorporated into the computer software have been described previously (11) and are presented in detail in the Appendix. The software was written in the C language by one of the authors (S.L.S). The pharmacokinetic algorithm in the software approximated the differential equations for drug transfer between compartments using Euler's numerical technique (11) with one iteration every 10 sec. The software adjusted the infusion rate every 10 sec to maintain the desired target concentration and recorded these rates on the hard disk to provide a precise record of the drug infusion.

### Study Outline

*Study 1*

Nine chronically instrumented rats received 20 mg/kg of thiopental as a 30- 60-sec infusion through an indwelling intravenous catheter in the jugular vein, and a tenth rat received 10 mg/kg via an otherwise identical infusion. Blood was sampled from an indwelling catheter in the abdominal aorta. Blood samples of 150–200 μl were gathered at approximately 0.5, 1, 1.5, 2.5, 4, 6, 9, 12, 20, 30, 45, 75, 150, and 180 min, relative to the start of the infusion (time 0). Plasma thiopental concentration was measured using an HPLC assay as described by Ebling *et al.* (13). Following each study, a sample of the thiopental infusate was also assayed by HPLC, and the actual dose of thiopental calculated. Following thiopental determinations, the parameters of a triexponential disposition function,

$$\text{Concentration } (t) = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t} \quad (1)$$

were fit to the pooled data using MKMODEL,[4] a non-linear least-squares regression program, as described below. These parameters were converted into the micro-rate constants of a three-compartment model for use by a CCIP in study 2.

*Study 2*

We used a CCIP to infuse thiopental to 11 rats through an indwelling intrajugular catheter. The infusion algorithm used by the CCIP incorporated the micro-rate constants derived in study 1. The computer was programmed to maintain arterial plasma thiopental concentrations of 5, then 20, and finally, 50 μg/ml (seven rats) or 10, then 40, and finally, 100 μg/ml (four rats). Each target level was maintained for 30 min. Arterial samples of 150–200 μl were drawn every 1–2 min initially at each target concentration, then at increasingly longer intervals, for approximately six samples per target concentration.

We used nonlinear regression to fit the parameters of the triexponential disposition function to the plasma concentrations observed in study 2. The nonlinear regression used the drug infusion scheme recorded by the CCIP during each study, along with the measured plasma thiopental concentrations. A pooled data population approach was used to fit all observations simultaneously to a single model, as described previously (4). These revised parameters were again converted to micro-rate constants for use by a CCIP.

*Study 3*

We used a CCIP to administer an intravenous thiopental infusion to seven rats. The infusion algorithm in study 3 used the revised thiopental micro-rate constants derived from study 2. The computer was programmed to maintain plasma thiopental concentrations of 5, then 20, and finally, 60 μg/ml (four rats) or 10, then 40, and finally, 100 μg/ml (three rats). Each target level was maintained for approximately 40 min. Plasma sampling and measurement of pump performance were as described for study 2.

### Data Analysis

The performance of the CCIP in study 2 and study 3 was measured in terms of the concentration predicted by the pharmacokinetic model ($C_P$). This measurement differed slightly from the target concentration ($C_T$) the CCIP was attempting to maintain. At the time of each study, the computer assumed that the thiopental concentration in the syringe was 25 mg/ml. However, we subsequently analyzed the thiopental concentration in each syringe and adjusted the predicted concentrations accordingly.

The performance of the computer-controlled infusion pump was measured using the median prediction error (MDPE), the median absolute prediction error (MDAPE), and the root mean squared error (RMS error), as described below.

We defined the performance errors as the differences between each measured concentration ($C_M$) and the concentration predicted by the pharmacokinetic model, $C_P$, weighted by $C_P$. We weighted the errors by $C_P$ because the magnitude of the errors tended to be proportional to $C_P$. Thus, the performance error (PE) for each observation was defined as

$$\text{PE} = \frac{C_M - C_P}{C_P} \times 100\% \quad (2)$$

The performance of the CCIP in each study was measured using the median *absolute* performance error (MDAPE):

$$\text{MDAPE} = \text{median}(|\text{PE}_1|, |\text{PE}_2|, \ldots, |\text{PE}_n|) \quad (3)$$

where $n$ is the total number of samples in the population. The MDAPE is a measure of the degree of inaccuracy of the measured vs targeted thiopental concentrations. This pooled data approach (i.e., $n$ = total number of samples in the study) was consistent with our pooled pharmacokinetic analysis, described below.

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The median performance error (MDPE) reflects the presence of systematic underdosing or overdosing by the CCIP. The MDPE was calculated as

$$\text{MDPE} = \text{median}(\text{PE}_1,\text{PE}_2, \ldots ,\text{PE}_n) \quad (4)$$

Because many pharmacokineticists are familiar with root mean squared (RMS) error, we also calculated the RMS performance error, RMSPE, as a measure of inaccuracy:

$$\text{RMSPE} = \sqrt{\frac{\sum_{i=1}^{n} \text{PE}_i^2}{n}} \quad (5)$$

The observed thiopental concentration-vs-time data from study 1 and study 2 were analyzed by MKMODEL, an extended least-squares nonlinear regression program. MKMODEL was modified to incorporate an infusion that changed every 10 sec, based on the equations of Maitre *et al.* (14). Thus, the drug input used by MKMODEL was the actual CCIP infusion scheme recorded by the computer during each study. We also modified MKMODEL to fit simultaneously the observations for all rats in each study to a single "best" estimate of the pharmacokinetic parameters for the entire population in that study, using a simple pooled data approach. We have previously described the use of MKMODEL to derive parameters from a pharmacokinetic study using a CCIP (4).

MKMODEL estimated the $\alpha$, $\beta$, and $\gamma$ hybrid rate constants, $V_c$ (the volume of the central compartment), $k_{21}$, and $k_{31}$. These parameters were chosen because they could be most rapidly converted into the additional parameters required by the equations derived by Maitre *et al.* (14). MKMODEL weighted the squared errors by the predicted variance, as required by extended least-squares nonlinear regression analysis. A constant coefficient of variation variance model (15) was employed:

$$\text{variance} = \sigma(C_P)^2 \quad (6)$$

The power term was fixed at 2 after an initial extended least-squares analysis suggested that 2 was close to the correct value. When the power term is fixed at 2, the $\sigma$-scaled weighted residual for each observation when using a constant coefficient of variation variance model was

$$\sigma\text{-scaled weighted residual} = \frac{C_M - C_P}{\sigma C_P} \quad (7)$$

Note that the only difference between the weighted performance error in Eq. (2) and the $\sigma$-scaled weighted residual in Eq. (7) is that Eq. (2) contains a scale factor of 100, to convert the error into a percentage, while Eq. (7) has a scale factor of $\sigma$, which is required for the extended least-squares objective function of MKMODEL. The $\sigma$-scaled weighted residual, which is a retrospective measure of goodness of fit of each observation, can be converted to a percentage by scaling the error by 100 rather than $\sigma$. This permits comparison of the weighted residual with the weighted performance error. We therefore defined the weighted residual for each observation as

$$\text{weighted residual} = \frac{C_M - C_P}{C_P} \times 100\% \quad (8)$$

Comparison of Eq. (8) with Eq. (2) shows that the weighted residuals, which are a measure of goodness of fit, are defined in exactly the same way as the performance errors, which are a prospective measure of CCIP accuracy. From the weighted residual we calculated the median absolute weighted residual (MDAWR),

$$\text{MDAWR} = \text{median}(|\text{WR}_1|,|\text{WR}_2|, \ldots ,|\text{WR}_n|) \quad (9)$$

the median weighted residual (MDWR),

$$\text{MDWR} = \text{median}\ (\text{WR}_1,\text{WR}_2, \ldots ,\text{WR}_n) \quad (10)$$

and the RMS weighted residual (RMSWR),

$$\text{RMSWR} = \sqrt{\frac{\sum_{i=1}^{n} \text{WR}_i^2}{n}} \quad (11)$$

It can be shown that the RMS weighted residual is the maximum-likelihood estimator of $\sigma$ (cf. the Appendix of Ref. 4).

Our prospective measures of CCIP performance [Eqs. (3)–(5)] are thus exactly analogous to our retrospective measures of goodness of fit of our pharmacokinetic model to the data [Eqs. (9)–(11)]. This facilitates comparison of CCIP performance, measured prospectively using MDAPE, MDPE, and RMSPE, with the residual variability after estimating the parameters of the pharmacokinetic model, measured retrospectively using MDAWR, MDWR, and RMSWR. This is an important comparison, because the weighted residuals represent the best possible performance of the model being used, given the underlying biologic variability.

## RESULTS

### Study 1

Figure 1 shows the plasma thiopental concentration-vs-time data for all 10 rats (circles). The solid lines show the concentrations predicted for each animal from the pharmacokinetic data analysis. The lines do not overlap because of differences in the thiopental dose administered to each animal. In all but one animal, the differences in dose resulted from slight differences in infusate thiopental concentration based on our subsequent assay of a sample of infusate. One animal received a markedly smaller dose than the other nine animals. The observed and predicted plasma thiopental concentrations for this animal can be easily distinguished below the cluster of curves and data points for the other nine animals. The estimated parameters for these rats are shown in Table I.

Figure 2 shows the weighted residuals for study 1, expressed as percentages, over time for the 10 rats. A few large positive errors in the first 10 min are balanced by a large number of small negative errors during this period. This negative bias diminishes over time. Table II shows the measures of performance (i.e., weighted residuals) for study 1. The MDAWR was 16%. The MDWR of −13% quantitates the negative bias seen in Fig. 2. The RMS error was 24%.

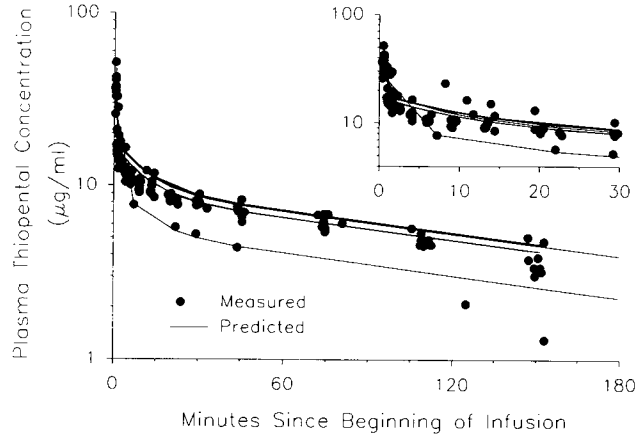


Fig. 1. Study 1: Thiopental plasma concentrations over time (filled circles) during and after a short intravenous infusion (10 rats). The straight lines are the predicted levels from the pharmacokinetic model derived from these rats.

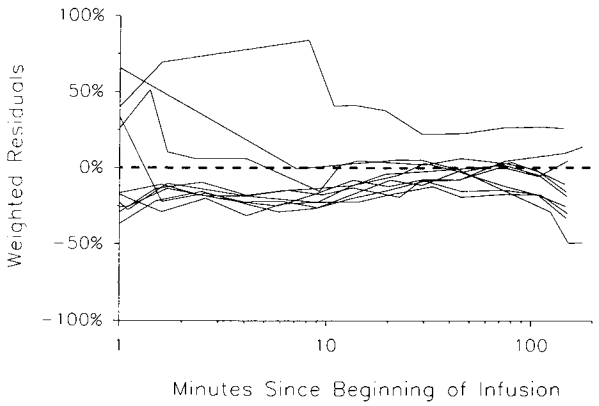


Fig. 2. Study 1: Weighted residuals over time for the pharmacokinetic model derived from a short intravenous infusion. A logarithmic time scale was used to permit visualization of the individual errors during the first few minutes.

## Study 2

Figure 3 shows the measured plasma thiopental concentrations over time for the first CCIP thiopental study. The dashed lines show the target concentrations, while the solid lines connect the individual observations in each rat. The pump was programmed with the parameters derived from study 1. Although the performance was fairly good, two problems can be seen. First, there was a uniformly positive bias (i.e., consistent overshoot) at each target concentration except the 100 μg/ml target concentration in group 2B. Second, the positive bias was particularly evident in the first few minutes, suggesting that the true volume of the central compartment was smaller than estimated in study 1.

Figure 4 shows the weighted performance errors for study 2. The strong positive bias can be seen, particularly in the first few minutes after each change in target concentration. Table II quantitates these observations. The MDAPE was 26%, while the MDPE was +25%. The RMS error was 42%.

New parameters for a three-compartment model were derived from the observations in study 2 using MKMODEL. This second parameter set is shown in Table I. Comparison of the parameters from study 1 and study 2 shows the smaller central and steady-state volumes of distribution estimated from study 2. Figure 5 shows the weighted residuals from the new model. Comparison of Fig. 5 with Fig. 4 shows the improvement obtained with the new parameter set. The improvement in accuracy (an entirely expected result) can be seen in Table II. The MDAWR for the second parameter set was 11%. The MDWR was 0%, and the RMS error was 16%.

Table I. Pharmacokinetic Parameters

| Parameter | Study 1 | Study 2 |
|---|---|---|
| Fractional coefficients (%) | | |
| $A$ | 83 | 82 |
| $B$ | 8 | 11 |
| $C$ | 9 | 7 |
| Hybrid rate constants ($min^{-1}$) | | |
| α | 4.33 | 3.07 |
| β | 0.097 | 0.125 |
| γ | 0.0049 | 0.0112 |
| Half-lives (min) | | |
| $t_{1/2}$ α | 0.16 | 0.23 |
| $t_{1/2}$ β | 7 | 6 |
| $t_{1/2}$ γ | 143 | 62 |
| Volumes ($L \cdot kg^{-1}$) | | |
| $V_1$ (central) | 0.183 | 0.122 |
| $V_2$ | 0.779 | 0.410 |
| $V_3$ | 0.910 | 0.784 |
| $V_{d_{ss}}$ | 1.87 | 1.32 |
| Clearances ($L \cdot min^{-1} \cdot kg^{-1}$) | | |
| $Cl_1$ (central) | 0.010 | 0.017 |
| $Cl_2$ | 0.60 | 0.25 |
| $Cl_3$ | 0.046 | 0.038 |
| Micro-rate constants ($min^{-1}$) | | |
| $k_{10}$ | 0.052 | 0.143 |
| $k_{12}$ | 3.31 | 2.08 |
| $k_{13}$ | 0.250 | 0.310 |
| $k_{21}$ | 0.777 | 0.620 |
| $k_{31}$ | 0.050 | 0.048 |

## Study 3

Figure 6 shows the results of the second CCIP study in

Table II. Measures of Performance

| Study | Type of measure | Parameter from Study 1 | | Parameter from Study 2 | |
|---|---|---|---|---|---|
| | | (Retrospective) | | | |
| Study 1 | Accuracy | MDAWR: | 16% | | |
| | Bias | MDWR: | −13% | Not calculated | |
| | Accuracy | RMSWR: | 24% | | |
| | | (Prospective) | | (Retrospective) | |
| Study 2 | Accuracy | MDAPE: | 26% | MDAWR: | 11% |
| | Bias | MDPE: | −25% | MDWR: | 0% |
| | Accuracy | RMSPE: | 42% | RMSWR: | 16% |
| | | | | (Prospective) | |
| Study 3 | Accuracy | | | MDAPE: | 15% |
| | Bias | Not calculated | | MDPE: | 0% |
| | Accuracy | | | RMSPE: | 19% |

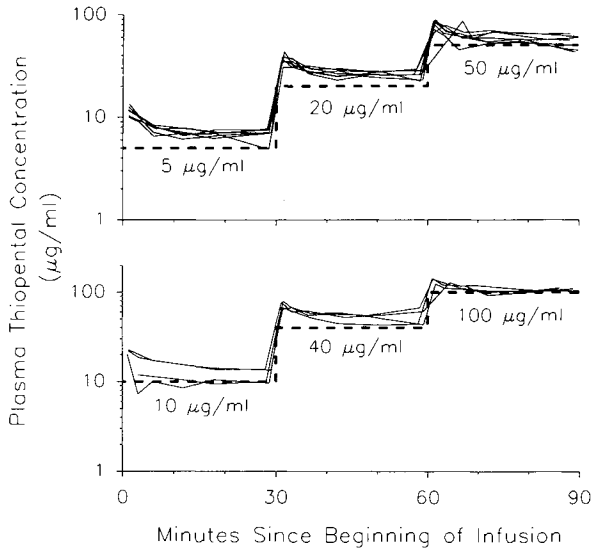


Fig. 3. Study 2: Thiopental plasma concentrations over time during a computer-controlled infusion (top, seven rats; bottom, four rats). The continuous lines connect the individual measured plasma concentration measurements. The dashed lines show the target concentrations.

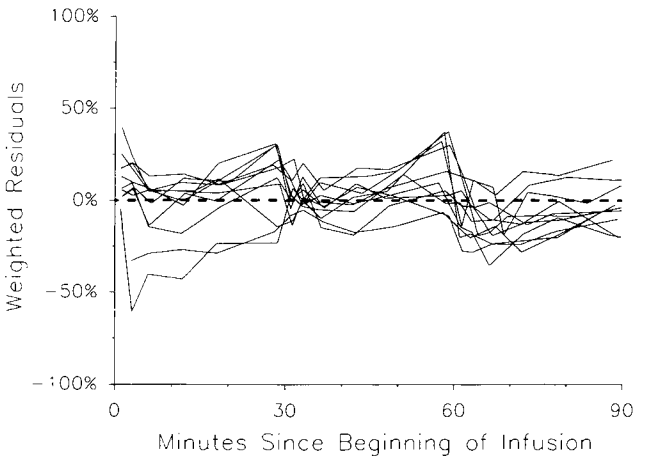


Fig. 5. Study 2: Calculated weighted residuals over time for the pharmacokinetic model derived from the thiopental concentrations measured in these 11 rats [Eq. (8)].

seven rats. Comparison of Fig. 6 with Fig. 3 shows the improvement in performance obtained when we used the thiopental pharmacokinetic parameters derived from study 2. Figure 7 shows the weighted performance errors. Comparison of Fig. 7 with Fig. 4 also shows the improved performance obtained with the second parameter set. As seen in Table II, the MDAPE was 15%, the MDPE was 0%, and the RMS error was 19%.

## DISCUSSION

Table II shows that the performance of the thiopental parameters derived from a CCIP study (study 2), as prospectively measured in study 3, was remarkably similar to the weighted residuals calculated from the data obtained in study 2. This demonstrates that pharmacokinetic studies using computer-controlled infusions can be used to derive pharmacokinetic parameters which can accurately attain and then maintain stable plasma drug concentrations.

The parameters derived from study 1 performed reasonably well at maintaining a steady concentration in study 2, suggesting that the pharmacokinetics of thiopental administered by a computer-controlled infusion pump are similar to the pharmacokinetics of thiopental following a brief zero-order infusion. The performance of the CCIP in study 3 was clearly superior to the performance of the CCIP in study 2. Comparison of the weighted residuals of the parameters estimated in study 2 (and tested in study 3) with the weighted residuals of the parameters estimated in study 1 (and tested in study 2) shows that there was less residual variability in the data following the computer-controlled administration than following the more conventional pharmacokinetic

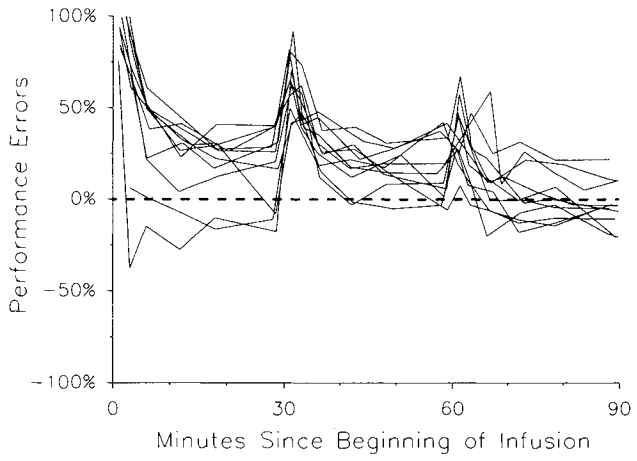


Fig. 4. Study 2: Calculated performance errors over time [Eq. (3)].

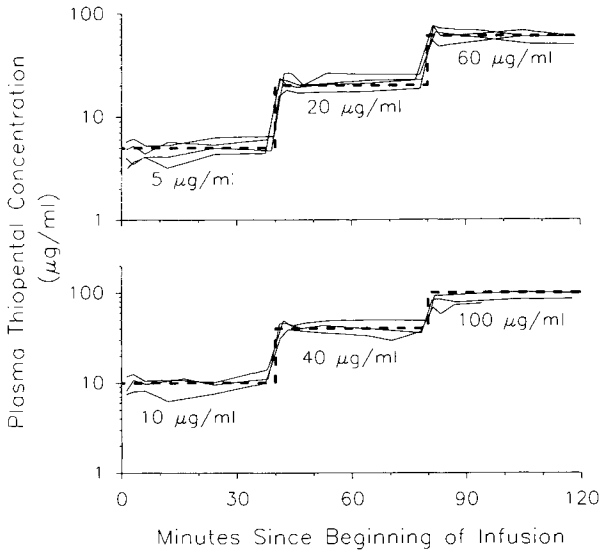


Fig. 6. Study 3: Thiopental plasma concentrations over time during a computer-controlled infusion (top, four rats; bottom, three rats). The continuous lines connect the individual measured plasma concentrations. The dashed line shows the target concentrations. The pharmacokinetic parameters in this study were derived from the observations in study 2.

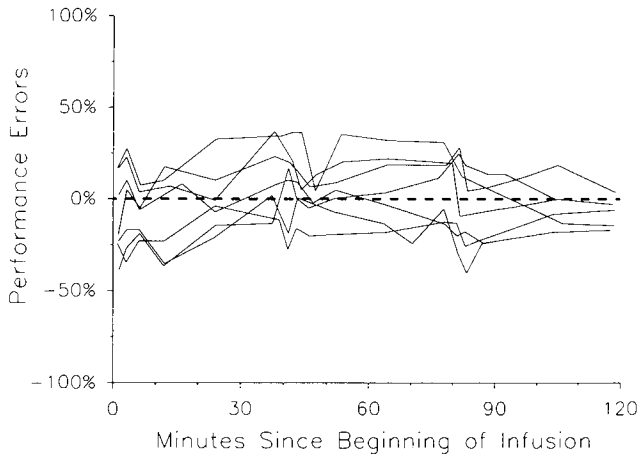


Fig. 7. Study 3: Calculated performance errors over time. Comparison with Fig. 4 shows improvement obtained when using thiopental pharmacokinetic parameters derived from a study in which thiopental was given by a CCIP.

study. Thus, noise in the data may have contributed to the comparatively worse performance of the parameters from study 1 when tested prospectively with a CCIP.

In study 2, we rapidly gathered thiopental samples every time the concentration was changed. The pump responds to each request for an increase in target concentration with a brief rapid infusion to raise the central compartment concentration. The plasma concentrations during and immediately following this brief, rapid infusion are determined primarily by the high-frequency (distribution-phase) pharmacokinetic components. We thus had three opportunities in each rat to sample the most rapid pharmacokinetic components. It is therefore not surprising that the parameters from study 2 prospectively performed better than the parameters from study 1 during the initial few minutes. The ability to sample the high-frequency pharmacokinetic components multiple times is not unique to computer-controlled infusion studies. It is entirely possible that a pharmacokinetic study with several separate boluses or infusions and rapid sampling of arterial blood could have characterized the high-frequency pharmacokinetic components (i.e., the early phase) and provided the excellent performance seen prospectively in study 3.

Figure 5 shows a pattern of positively biased errors at the times of changes in target concentrations. This suggests a small amount of model misspecification at the times of each change in plasma thiopental concentration. Additionally, Fig. 6 shows that at a target concentration of 100 μg/ml, the CCIP systematically maintained levels less than the target. The protein binding of thiopental has been shown to be saturable at high concentrations (16), and this may account for the apparent deviation from linear pharmacokinetics observed at the highest target.

The results of this study are consistent with our previous work demonstrating that fentanyl pharmacokinetic parameters from computer-controlled infusions can generate robust estimates of fentanyl pharmacokinetics in humans (4). That study showed that the pharmacokinetics of fentanyl, when administered by a CCIP, were similar to the disposition of fentanyl when given by a more conventional zero-order infusion.

The pharmacokinetic parameters shown in Table I reflect the limited duration of sampling in these studies. Thus, the terminal half-life from study 2 was only 62 min. This is likely to be an artifact of the 90-min duration of study 2. If blood had been sampled for a longer period of time, a longer elimination half-life may have been observed. Thus, the parameters shown in Table I would not necessarily provide good performance in a CCIP after 90 min. It is our opinion that it is unreasonable to expect good performance by a CCIP beyond the time period samples in the original study that provided the pharmacokinetic parameters.

The ability of computer-controlled infusions to reach rapidly and maintain stable plasma concentrations has important implications for basic research and clinical medicine (1–6). As a research tool, the CCIP provides a means to "clamp" plasma concentrations at steady levels, provided the pharmacokinetics programmed into the pump are accurate. Once the concentrations in the plasma are "clamped" at a known concentration, all tissues in the body will equilibrate with the plasma over time. For example, the half-time of equilibration between the plasma and the site of thiopental effect on the EEG is 1–2 min (17). We have previously demonstrated in human subjects that achieving a pseudo-steady-state plasma thiopental concentration with CCIP leads to a pseudo-steady-state concentration in the effect-site concentration following an adequate period for equilibration (18).

Once body tissues have equilibrated with the stable plasma drug concentrations made possible by a CCIP, it becomes possible to perform measurements of drug effect without the confounding influence of changing plasma and tissue drug concentration. The ability to clamp plasma drug concentrations accurately and reproducibly in small laboratory animals has not been demonstrated previously. We anticipate that this technique will find application in characterizing the concentration–response relationship for many drugs which can be intravenously administered.

## APPENDIX: PRINCIPLES OF THE COMPUTER-CONTROLLED INFUSION PUMP

A detailed appendix on the mathematical methods of the computer-controlled infusion pump can be obtained from L.L.G. or S.L.S.

The CCIP maintains a constant plasma drug concentration using a modification of the algorithm initially proposed by Schwilden (19). First, the target concentration ($C_T$) is instantaneously achieved in the plasma by administering a bolus of amount $C_T V_1$. Concurrent with the bolus, an infusion is started to maintain the desired target concentration, $C_T$, at the rate $I(t) = C_T V_1(k_{10} + k_{12}e^{-k_{21}t} + k_{13}e^{-k_{31}t})$. In this equation $t$ is the time from the initial bolus, $V_1$ is the volume of the central compartment, and $k_{10}$, $k_{12}$, $k_{13}$, $k_{21}$, and $k_{31}$ are the elimination and distribution rate constants, as shown in Fig. 8.

Figure 9 shows the actual infusion rate from one of the rats in study 2. This illustrates the initial rapid infusion required to step up (nearly) instantaneously to a higher concentration, as well as the exponentially declining infusion required to maintain the target concentration. Figure 9 also illustrates the reasons Schwilden's algorithm had to be modified for use in our CCIP.

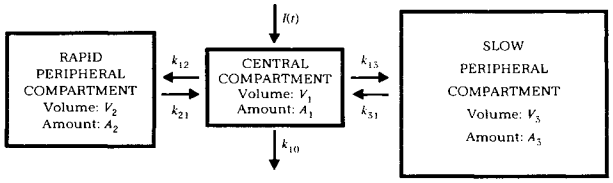


Fig. 8. Three-compartment pharmacokinetic model used by the computer-controlled infusion pump.

(1) To be useful in research, a CCIP must provide the ability to raise and lower the target concentration. As seen in Fig. 9, in the current study we desired three successive target concentrations. Schwilden's algorithm for achieving and maintaining a target concentration, as shown in the first paragraph above, assumes that there is no drug in the body at time 0.

(2) Schwilden's algorithm requires a continuously changing infusion rate. Commercially available infusion pumps can provide only constant-rate (i.e., zero-order) infusions. The exponentially declining infusion, which can be seen in Fig. 9, is approximated by changing the infusion rate at regular intervals (e.g., every 10 sec in the case of STANPUMP). This requires a discrete, rather than a continuous, time frame.

(3) The infusion pump may not give the exact dose requested by the computer because of limitations in the precision of data transmission between the infusion pump and the computer, the acceleration/deceleration delay in the pump mechanism, and the use of stepper motors which can infuse drug only in discrete quanta over time which may not exactly match the desired rate. Since the pump may not give the exact dose required, the computer needs to monitor continuously the amount of drug actually given and adjust each subsequent infusion accordingly. The fluctuations in the infusion rate between 10 and 30 min are an example of the computer continuously adjusting for small discrepancies between desired and actual infusion rates.

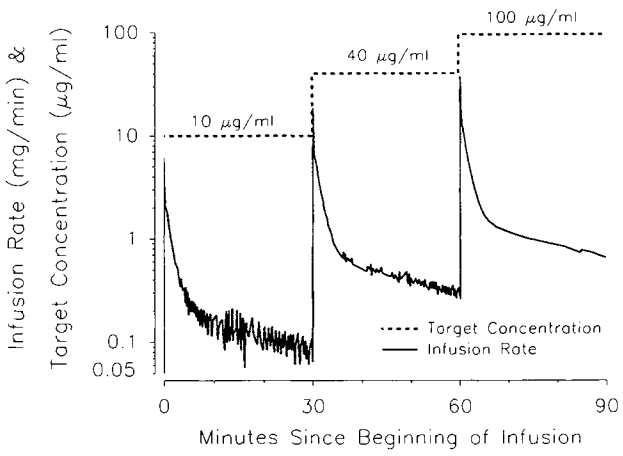


Fig. 9. The infusion rate required to achieve the target concentration in one rat from study 2. The dashed lines show the target concentration, while the continuous line shows the initial rapid rate required to reach each target concentration and the exponentially declining rate required to maintain each target concentration.

We made two modifications of the algorithm proposed by Schwilden. First, we used a discrete time frame, and second, the state variables (cf. A1, A2, and A3 in Fig. 8) were used to calculate the infusion rate required to achieve $C_T$. This modification has been described in detail (14) and analytical solutions have been reported by Maitre *et al.* (20), Jacobs (21), and Bailey and Shafer (22).

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