The role of metabolism, relative to redistribution, in the termination of anesthesia was examined in patients receiving a single bolus iv injection of thiopental. Additionally, it was determined if nonlinear protein binding occurs immediately after the bolus iv injection of thiopental, possibly enhancing thiopental effect. Thiopental pharmacokinetics and protein binding were determined in 12 surgical patients with normal hepatic function. Using the pharmacokinetic equations listed in the appendix, plasma concentration over time data were used to quantitate the contribution of metabolism to the early decline of thiopental plasma concentrations after a single iv bolus administration. The fraction of thiopental loss from the central compartment due to metabolism was calculated to be 0.14 ± 0.06 (mean ± SD) at 1 min and 0.18 ± 0.04 at 15 min. These data confirm that metabolism is far less important than distribution in the initial decline of blood and brain concentrations of thiopental, and, therefore, termination of thiopental anesthetic effect. The protein binding of thiopental from 0.5 to 15 min was found to be linear over a concentration range of 33 ± 60 μg/ml to 8.9 ± 6.2 μg/ml. Thus, concentration-dependent or nonlinear protein binding of thiopental after a single iv bolus administration could not be demonstrated and does not enhance thiopental anesthetic effect. (Key words: Anesthetics, intravenous: thiopental. Metabolism: thiopental. Pharmacokinetics: distribution; metabolism. Protein: binding.)

THIOPENTAL has had a major role in clinical anesthesia since its introduction in 1954. Originally, the ultrashort action of thiopental was attributed to rapid metabolism. It was not until 1952 that Brodie, Bernstein, and Mark first appreciated redistribution of thiopental from the plasma and brain to less well-perfused tissues of the body. They concluded that fat was the important depot influencing early termination of thiopental effect. In 1960, Price used a digital computer to simulate distribution in the body and concluded that the early decrease of the thiopental brain concentration was due primarily to redistribution to muscle, not fat, because of the relatively greater mass and blood flow of muscle. Price ignored the contribution of hepatic metabolism in his simulation of thiopental disposition. In 1966, Saidman and Eger extended Price’s physiologic model by adding hepatic metabolism, concluding that hepatic metabolism was important in the initial decline of blood and brain concentrations of thiopental, and therefore the termination of thiopental anesthetic effect. We have reexamined metabolism versus redistribution in the termination of thiopental anesthetic effect by gathering serum concentration data in individual patients to accurately characterize thiopental distribution and elimination. Pharmacokinetic concepts were used to quantitate the contribution of metabolism versus redistribution in the initial decline of thiopental serum concentration after an intravenous bolus administration.

The degree of protein binding is also important in the clinical use of thiopental, because it is the free, unbound drug that penetrates the blood-brain barrier to the brain and the cardiovascular system, causing drug effect and possible toxicity. Morgan et al. recently reported that at thiopental plasma concentrations between 100 and 150 μg/ml, the protein binding of thiopental decreased from 85% to 70%, indicating that thiopental protein binding is nonlinear. If this is true, thiopental plasma concentrations greater than 100 μg/ml should increase anesthetic and toxic effects, because a greater fraction of the thiopental will be free to cross the blood-brain barrier. We determined the protein binding of thiopental in arterial samples obtained immediately after bolus iv administration of thiopental to demonstrate the actual arterial serum concentrations achieved and the presence or absence of nonlinear thiopental protein binding.

Methods

Subjects and Protocol

The protocol was approved by the Institutional Human Studies Committee and informed consent was obtained from 12 patients (three women and nine men) with normal hepatic function, with a mean age (±SD) of 35.9 ± 12 years, and a mean weight of 72.6 ± 11 kg. They were all scheduled for minor surgical procedures using general anesthesia, and did not sustain significant blood loss. Each patient was administered a single, rapid, iv bolus of 6.0 ± 0.74 mg/kg thiopental over 5 s for induction of anesthesia. Enfutane 1–2% in nitrous ox-
ide (70%), was used to maintain anesthesia. Samples of blood were obtained from a radial artery catheter for the first 2 h, then venous samples were collected for the remainder of the study. Blood samples were obtained at 0.5, 1, 2, 3, 5, 7, 10, 15, 20, 30, and 45 min, and 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h. This sampling protocol allowed accurate characterization of the peak thiopental plasma concentration and the distribution and elimination phases.

ANALYTICAL TECHNIQUES

A high performance liquid chromatography assay sensitive to 25 ng/ml was used to measure total thiopental concentrations. The free fraction (unbound fraction) of thiopental was determined on the arterial samples obtained at 0.5 to 15 min using an Amicon MPS-1® micropartition ultrafiltration system (Amicon Corp., Lexington, Massachusetts), with a YB-30 membrane. This membrane does not bind thiopental in a nonspecific manner. Thiopental concentration in the ultrafiltrate was measured with the above assay. If total thiopental serum concentrations did not exceed 100 µg/ml, blank serum samples for those patients were spiked to 100 and 150 µg/ml, and the extent of protein binding was determined. The ultrafiltrate represented about 20% of the serum volume. This volume of ultrafiltrate should not affect the interpretation of the protein binding measurement. No protein was detectable in the ultrafiltrate with a protein assay sensitive to 1 µg/ml (Bio-Rad Protein Assay). To validate the above ultrafiltration technique, we analyzed with equilibrium dialysis the protein binding of the same serum sample spiked to 50 µg/ml, 100 µg/ml, and 150 µg/ml. A Spectrum® dialysis apparatus (Spectrum Medical Industries, Inc., Los Angeles, California) was used with Spectra Por-2 (12,000–14,000 M.W. cutoff) membranes. The dialysis was performed at 37°C for 24 h, an adequate time to achieve equilibrium. Serum (1 ml) samples were dialyzed against sterile Krebs buffer (1 ml), pH 7.4, using Teflon® dialysis cells. Preliminary experiments showed that thiopental did not bind to this membrane. Each sample was analyzed in duplicate.

DATA ANALYSIS

Total thiopental serum concentration versus time data for each patient were fit to a triexponential equation interpreted as a three-compartment mammillary model (fig. 1) using nonlinear least-squares regression. When the same data were fit to a biexponential equation (two-compartment model), statistical testing on the residual sum of squares demonstrated a significant preference for the triexponential model. A weighing value of 1/x² was used. From the triexponential fit, the rapid and slow distribution half-lives, elimination half-life, volume of the central compartment, volume of distribution at steady state, and clearance were determined using standard pharmacokinetic formulas.

To determine the contribution of metabolism to the termination of thiopental effect, we used a method suggested by Dr. Lewis B. Sheline (personal communication). We calculated the total cumulative amount of thiopental lost from the central compartment due to metabolism for frequent time intervals using the following equation:

\[
\text{metabolic loss} = C_l \times \int_0^t \text{Cp} \, dt
\]

where \( \text{Cp} \) = the serum concentration of thiopental at time \( t \) and \( C_l \) = total body clearance.

The amount of thiopental lost from the central compartment due to both metabolism and distribution (total loss) to the peripheral compartments was calculated for the same time intervals using the equation

\[
\text{total loss} = V_1 \times [\text{Cp}(0) - \text{Cp}(t)]
\]

where \( \text{Cp}(0) \) = the serum concentration of thiopental at \( t = 0 \); \( \text{Cp}(t) \) = the serum concentration of thiopental at time \( t \); and \( V_1 \) = volume of the central compartment.

The derivation of equations 1 and 2 can be found in the Appendix.

Values obtained from these equations were used to form the fraction (metabolic loss/total loss) to measure the amount of thiopental lost over time from the central compartment due to metabolism. The change of central compartment thiopental concentration should reflect changes in brain concentration, which governs the termination of thiopental effect, because the central compartment of pharmacokinetic models usually is considered to be composed of the vessel-rich organs.
administration. The degree of thiopental protein binding versus time for 0.5 to 15 min was used for each subject. The Mann-Whitney U-test ($P < 0.05$) was used to compare the degree of protein binding in the blood samples whose concentrations were 100 and 150 μg/ml.

**Results**

The thiopental serum concentration decay curve for a representative patient is shown in figure 2. It is characterized by a triexponential equation representing a three-compartment model (fig. 1). The pharmacokinetic parameters that characterize thiopental's distribution and elimination in the body are shown in table 1. The fraction of thiopental lost from the central compartment from 1 to 15 min is illustrated in figure 3. At 1 min, the fraction of the administered dose removed from the central compartment by metabolism was 0.14 ± 0.06 (SD). At 15 min, this fraction was increased to only 0.18 ± 0.04. Extending the time axis to 4,000 min (67 h) in figure 4 illustrates how long it takes before the fraction approaches 1.0, representing complete metabolic loss of thiopental from the body.

The concentration range of thiopental in arterial blood for the first 15 min after a single bolus iv admin-

![Graph showing thiopental serum concentration decay curve.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931438/)
Fig. 3. The fraction of thiopental lost from the central compartment due to metabolism from 1 to 15 min is illustrated (mean ± SD). This time frame represents the dissipation of the clinical effect of a single iv bolus injection of thiopental. The fraction at 1 min is 0.14 ± 0.06, and at 15 min is only 0.18 ± 0.04.

Fig. 4. The fraction of thiopental lost from the central compartment due to metabolism from time 0 to 4,000 min (mean ± SD). At 4,000 min (67 h), the value of the fraction approaches 1.0 (complete metabolic loss from the body).

...istration is shown in figure 5. Thiopental serum concentration at 0.5 min was 93 ± 60 μg/ml, and by 15 min had declined to 6.9 ± 0.62 μg/ml. The peak serum concentration of thiopental at 0.5 min exceeded 100 μg/ml in only five of the 12 patients. The linear regression line for the mean per cent protein binding at each arterial sample collection time is shown in figure 6. The 95% confidence limits of the slope included zero, indicating that there is no significant change of thiopental protein binding with time as the plasma concentration rapidly declines. When the thiopental protein bindings at 100 and 150 μg/ml were statistically compared, no significant difference was found. Table 2 compares the degree of protein binding in the same serum sample at three thiopental concentrations, using the two methods of protein binding measurement. Both techniques gave very similar values. Because of the small sample size, a statistical analysis was not performed.

**Discussion**

Thiopental distribution and elimination in the body has been modeled physiologically. However, these models are based upon computer simulations that use...
presumed values of organ blood flow, organ volume, thiopental tissue/blood partitioning, and hepatic extraction. These values generally cannot be measured in any one patient; therefore, these models are not useful to characterize an individual patient. Recently, several authors have described the distribution and elimination of thiopental in the body and pharmacokinetic models using plasma concentration versus time data which allow one to characterize an individual patient. Our pharmacokinetic study was designed to optimally characterize the distribution phases of thiopental with frequent, early, arterial sampling after iv bolus administration. Early frequent sampling demonstrates a triexponential behavior with a rapid and slow distribution phase and a terminal elimination phase. The rapid distribution phase may represent equilibration between the vessel-rich group and muscle tissue, while the slow distribution phase may represent equilibration between the vessel-rich group and fat tissue. Exact anatomic or physiologic interpretation of these distribution phases is not possible, however, without actually sampling the tissues and measuring thiopental tissue concentrations.

Our pharmacokinetic data compare favorably with other studies. The terminal elimination half-life determined in this study (mean ± SD of 12.0 ± 5.5 h) is comparable with other reported half-lives. It is longer than the half-life (mean ± SD of 5.14 ± 0.74 h) reported by Ghoneim and Van Hamme, because we sampled for 24 h, while Ghoneim and Van Hamme collected samples for only 12 h. Because thiopental has a low hepatic extraction ratio, it exhibits capacity-dependent elimination; elimination depends upon the intrinsic hepatic enzyme activity and the degree of plasma protein binding. This results in a relatively low clearance value. The moderately large apparent volume of distribution at steady state results from the extensive distribution of thiopental into fat tissues.

Calculation of the fraction metabolic loss/total loss represents an attempt to measure the contribution of metabolism in the termination of the anesthetic effect of thiopental, addressing an issue raised by Saltman and Eger over 15 years ago. Our pharmacokinetic approach is based upon the assumption that thiopental central compartment levels approximate brain concentrations. During the first minute after thiopental bolus administration, the fraction of thiopental lost from the central compartment due to metabolism was 0.14. This represents the initial hepatic uptake and subsequent metabolism of thiopental. It correlates well with a hepatic extraction ratio of approximately 0.15 (range of 0.12 to 0.19) that can be calculated from our clearance values and from those of other investigators.

Over the next 15 min, when the central nervous system effect of thiopental is dissipating, the fraction of the thiopental dose lost from the central compartment due to metabolism increased from 0.14 ± 0.06 to only 0.18

Fig. 5. Serum concentration (mean ± SD) of thiopental in arterial blood for the first 15 min after a single bolus iv dose of thiopental for 12 patients.

Fig. 6. Per cent protein binding of thiopental (mean ± SD) for each patient's arterial sample collection time. The solid line is the linear regression of all the samples from 12 patients. The 95% confidence limits of the slope of this line includes zero, indicating that nonlinear or concentration-dependent protein binding of thiopental does not occur in the time-frame from 0.5 to 15 min after iv bolus injection.
± 0.04. This indicates that most of the change in thiopental serum concentration immediately after the bolus iv injection is secondary to movement of drug from the central compartment to the two peripheral compartments. This suggests that metabolism is not a major contributor to the early decline of central compartment thiopental levels immediately after a bolus injection. The role of metabolism would become more relevant with repeated or very large doses of thiopental. Assuming no alteration of brain sensitivity, protein binding, or redistribution of thiopental to tissue stores, changes in overall thiopental clearance by enzyme induction or hepatic dysfunction alone should have little impact on the termination of thiopental anesthetic effect.

Central nervous system and cardiovascular depression could be enhanced if nonlinear or concentration-dependent decreases of thiopental protein binding occurred at peak plasma concentrations after an iv bolus administration of thiopental. Morgan et al.6 predicted a significant decrease in protein binding of thiopental at serum concentrations above 100 μg/ml. However, because of some study design problems, Morgan et al.6 were not able to determine if this phenomenon was relevant in the clinical use of thiopental. First, they used venous samples, which may underestimate the peak arterial levels seen by the brain and the heart. Second, they waited 2 min after bolus iv administration of thiopental to obtain the first blood sample, and, therefore, they may have missed the highest thiopental plasma concentrations. They reported peak thiopental serum concentrations less than 15 μg/ml, and thus were unable to show that the plasma concentration of thiopental was ever high enough to approach the nonlinear portion of the protein binding curve.

In our study, only five of 12 of the patients had peak arterial thiopental serum concentrations exceeding 100 μg/ml in the first sample obtained at 0.5 min. In all patients studied, protein binding was linear with time from 0.5 to 15 min after a single iv bolus injection of thiopental. Additionally, even blank serum samples that spiked to 100 and 150 μg/ml thiopental did not demonstrate nonlinear protein binding. Although both our study and the study of Morgan et al.6 used ultrafiltration validated with equilibrium dialysis to quantitate the degree of protein binding, different membranes and buffers were used. We conclude that serum protein binding of thiopental is not significantly concentration-dependent over the usual therapeutic range of plasma levels seen in patients who are given a dose of thiopental adequate for induction of anesthesia.

Our data demonstrate two aspects important in the pharmacology of thiopental anesthetic effect. First, metabolism is far less important than redistribution in the termination of thiopental anesthetic effect. Second, concentration-dependent decreases of thiopental protein binding were not demonstrated.

The authors thank Mrs. S. Harapat for assay development and analysis, and Ms. F. Buran for preparation of this manuscript.

### Table 2. A Comparison of Equilibrium Dialysis and Ultrafiltration

<table>
<thead>
<tr>
<th>Thiopental Concentration (μg/ml)</th>
<th>Equilibrium Dialysis (% Bound)</th>
<th>Ultrafiltration (% Bound)</th>
</tr>
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<tr>
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<tr>
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<tr>
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<tr>
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<td><strong>Coefficient of variation</strong></td>
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<td><strong>1.5%</strong></td>
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</table>

### References

APPENDIX

The following derivation is from Dr. Lewis B. Sheiner (personal communication): If a drug is eliminated from the body only by hepatic metabolism (as is the case for thiopental) the pharmacokinetic term, clearance (Cl), characterizes the ability of the body to metabolize the drug. The amount of drug that is metabolized from the central compartment (metabolic loss, ML) over the time (t) interval $t_1$ to $t_2$ is approximated by:

$$ML = Cl \times \int_{t_1}^{t_2} Cpdt$$

(3)

At early times after a bolus dose, ML will actually be less than that given by equation 3 because the drug first must distribute into the clearing organs (liver and/or kidney) before it actually can be metabolized. The ML computed from equation 3 is therefore an upper bound for the time periods of interest.

The amount of total drug loss (TL) from the central compartment (metabolism plus distribution to peripheral compartments) over the time interval $t_1$ to $t_2$ is defined by:

$$TL = V_1(Cp_{t_1}) - Cp_{t_2}$$

(4)

where $V_1$ = volume of the central compartment; and $Cp_{t_1}$ and $Cp_{t_2}$ are serum concentrations at times $t_1$ and $t_2$.

Therefore, the fraction metabolic loss/total loss from the central compartment is defined by:

$$\frac{ML}{TL} = Cl \times \frac{\int_{t_1}^{t_2} Cpdt}{[V_1 \cdot Cp_{t_1} - Cp_{t_2}]}$$

(5)

The $Cp$ vs $t$ data for each patient is characterized by the following equation:

$$Cp_{t_1} = \sum_{i=1}^{n} A_i e^{-\alpha t_i}$$

(6)

where $A_i$ = intercept of the ln Cp vs t curve at $t = 0$; $\alpha$ = rate constant characterizing the decline of Cp; and $n$ = number of exponents necessary to characterize the data.

When equation (6) is substituted into equation (5) to calculate Cl, $Cp_{t_1}$, $Cp_{t_2}$, and $V_1$ the following equation is obtained:

$$\frac{\sum_{i=1}^{n} A_i / \alpha_i}{\sum_{i=1}^{n} A_i / \alpha_i}$$

(7)