Modifications of Blood Volume Alter the Disposition of Markers of Blood Volume, Extracellular Fluid, and Total Body Water

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ABSTRACT
Recirculatory pharmacokinetic models for indocyanine green (ICG), inulin, and antipyrine describe intravascular mixing and tissue distribution after i.v. administration. These models characterized physiologic marker disposition in awake, spleenectomized dogs while they were normovolemic, volume loaded (15% of estimated blood volume added as a starch solution), and mildly and moderately hypovolemic (15 and 30% of estimated blood volume removed). ICG-determined blood volumes increased 20% during volume loading and decreased 9 and 22% during mild and moderate hypovolemia. Dye (ICG) dilution cardiac output (CO) increased 31% during volume loading and decreased 27 and 38% during mild and moderate hypovolemia. ICG-defined central and fast peripheral intravascular circuits accommodated blood volume alterations and the fast peripheral circuit accommodated blood flow changes. Inulin-defined extracellular fluid volume contracted 14 and 21% during hypovolemia. Early inulin disposition changes reflected those of ICG. The ICG and inulin elimination clearances were unaffected by altered blood volume. Neither antipyrine-defined total body water volume nor antipyrine elimination clearance changed with altered blood volume. The fraction of CO not involved in drug distribution had a significant effect on the area under the antipyrine concentration-versus-time relationships (AUC) in the first minutes after drug administration. Hypovolemia increased the fraction of CO represented by nondistributive blood flow and increased the antipyrine AUC up to 60% because nondistributive blood flow did not change, despite decreased CO. Volume loading resulted in a smaller (less than 20%) antipyrine AUC decrease despite increased fast tissue distributive flow because nondistributive flow also increased with increased CO.

In a 10-year survey (1967–1976) of mortality associated with 240,483 anesthetics, Harrison (1978) found that the induction of anesthesia in hypovolemic subjects was the most common cause of death attributed to anesthesia and of an unknown amount of anesthetic-associated complications. The challenge of providing general anesthesia for hypovolemic patients is well recognized (Graves, 1974) and is perhaps best illustrated by the tragic consequences of the administration of thiopental to the hypovolemic casualties of the Japanese attack on Pearl Harbor (Halford, 1943).

Price (1960) explained the increased reactivity of hypovolemic patients to drugs such as thiopental on the basis of the increased percentage of cardiac output (CO), hence drug delivery, received by both the brain and the myocardium in the hypovolemic state. Subsequent studies have confirmed Price’s prediction of increased reactivity to drugs and increased plasma drug concentrations in hypovolemic subjects. Benowitz et al. (1977) found significantly increased arterial plasma lidocaine concentrations after an i.v. bolus dose in monkeys subjected to 30% exsanguination, which they attributed to decreases in both initial and steady-state volumes of distribution and elimination clearance (ClP). In addition, using data from studies in monkeys, they predicted marked increases in brain lidocaine concentrations after drug administration to a human after 30% hemorrhage. Decreases in

ABBREVIATIONS: CO, cardiac output; AUC, area under the blood concentration-versus-time relationship; Hct, hematocrit; ICG, indocyanine green; PA, pulmonary artery; MAP, mean arterial pressure; MTT, mean transit time; VC, central volume; VT-F, pulmonary tissue volume; VNDS, fast nondistributive peripheral pathway volume; CHNDS, clearance to the fast nondistributive peripheral pathway; VTS, slow nondistributive peripheral pathway volume; CIT, clearance to the slowly (fast) equilibrating tissue volume; CT, clearance to the rapidly (fast) equilibrating peripheral tissue compartment; VTS, slowly equilibrating tissue volume; CL, elimination clearance; VSS, total (steady-state) volume of distribution; ΣCl, total (sum of) all (peripheral and elimination) clearances; LSD, least significant difference.

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anesthetic requirements for both thiopental (33%) and ketamine (40%) in the pig after 30% blood loss (Weiskopf and Boget, 1985) were nearly identical with those predicted by the perfusion model of Price (1960). Increased reactivity of the hypovolemic dog to midazolam has also been reported by Adams et al. (1985), with increased arterial plasma midazolam concentrations yet no detected changes in the volumes of distribution.

Price (1960) also predicted that patients with increased blood flow to indifferent tissues such as muscle and portal tissues (e.g., thyrotoxic or apprehensive patients) would require larger doses of thiopental because a smaller fraction of the i.v. administered drug would appear in the brain. Data from a study of lidocaine disposition during an isoproterenol infusion in a single rhesus monkey (Benowitz et al., 1977) are consistent with this prediction; the isoproterenol infusion apparently increased the initial volume of distribution in a two-compartment model of lidocaine disposition by 16% and increased lidocaine $C_l$ by 40%.

Factors affecting the early arterial drug concentration-versus-time profile influence the intensity and timing of the onset of drug effect for rapidly acting i.v. anesthetics such as thiopental (Sheiner et al., 1981); these factors include intravascular mixing, pulmonary uptake, and distribution to highly perfused tissues by blood flow and transcapillary diffusion (Riggs, 1963; Krejcie et al., 1997). Our recently developed recirculatory pharmacokinetic model describes these processes by referencing them to the disposition of markers of intravascular space, extracellular fluid space, and body water (Krejcie et al., 1996a; Avram et al., 1997). Indocyanine green (ICG) binds to plasma proteins rapidly and completely, impeding its extravascular distribution (Henthorn et al., 1992). The polysaccharide inulin distributes from intravascular space to interstitial fluid by free water diffusion through aqueous endothelial fenestrations (Henthorn et al., 1982; Krejcie et al., 1996a). Antipyrine, a marker of total body water (Soberman et al., 1949) including pulmonary extravascular water (Brigham et al., 1971), distributes to a volume as large as total body water in a blood flow-dependent manner in many tissues and thus is a prototype for many lipophilic drugs (Renkin, 1952; Krejcie et al., 1996a).

The purpose of the present study was to examine the relationship of the cardiovascular and systemic effects of mild and moderate hypovolemia and volume loading to the distribution of drugs through mixing, flow, and diffusion.

**Materials and Methods**

**Experimental Protocol.** The design of this pharmacokinetic study entailed 16 individual experiments. Four purpose-bred male coonhounds, weighing 25 to 28.5 kg (26.6 ± 1.9 kg; Table 1), were studied on four occasions each in this Institutional Animal Care and Use Committee-approved study.

Approximately 1 month before being studied, the dogs were surgically prepared while under isoflurane anesthesia. The tip of a Vascular-Access-Port catheter (Access Technologies, Skokie, IL) was positioned near the aortic bifurcation via a femoral artery of each dog, and the access port was secured to the muscle fascia of the upper hind leg to facilitate frequent percutaneous arterial blood sampling (Garner and Laks, 1985). A splenectomy was also performed to prevent autotransfusion during acute hypovolemia (Carniero and Donald, 1977). The dogs were allowed to recover from surgery for at least 4 weeks before being studied for the first time.

All dogs were studied when normovolemic (control), when a targeted 15 and 30% of their calculated blood volume had been removed acutely (mild and moderate hypovolemia, respectively) and when acutely volume loaded with a starch solution targeted to equal 15% of their calculated blood volume (volume-loaded). The order in which these studies were conducted in each dog was randomized using a repeated measures Latin square experimental design. Studies were conducted at intervals of not less than 4 weeks. The dogs were trained for several weeks to lie in the left lateral decubitus position for these awake studies.

For all studies, after an overnight fast during which the dog was allowed water ad libitum, it was brought to the laboratory and positioned. When it was determined that the dog was calm and cooperative, the neck was prepped and the skin overlying the right external jugular vein was infiltrated liberally with 2% chloroprocaine. Using a modified Seldinger technique, an 8 Fr percutaneous shunt introducer was inserted into the external jugular vein. If the insertion could not be accomplished in a timely and humane fashion, the study was abandoned and rescheduled.

Arterial access for blood sampling by roller pump or syringe was achieved by inserting a 20-gauge Huber needle percutaneously in the Vascular-Access-Port; this also allowed systemic arterial blood pressure to be monitored via a solid-state pressure transducer (Trantec; Baxter-Edwards, Irvine, CA). A flow-directed thermal dilution pulmonary artery (PA) catheter (Baxter-Edwards 93A-140-7P, with a 20-cm proximal port) was inserted through the sheath introducer, positioned, and secured. The PA catheter was subsequently used to determine thermal dilution CO as well as to facilitate right atrial administration of the physiological markers. The side arm of the sheath introducer was used for maintenance fluid administration and the readministration of autologous blood. Hydration was maintained throughout the study by an infusion of 0.9% saline at a rate of

**TABLE 1**

<table>
<thead>
<tr>
<th>Subject characteristics and global pharmacokinetic parameters</th>
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<th>VSS</th>
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* | Significantly different from normovolemic control (p < .05), as determined by Fisher’s LSD test.
* | Significantly different from volume loaded (p < .05), as determined by Fisher’s LSD test.
* | Significantly different from mildly hypovolemic (p < .05), as determined by Fisher’s LSD test.

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3 ml/kg/h. Once the study was well under way, a lubricated 7 Fr single-lumen balloon-tipped catheter was inserted through the urethra into the bladder to facilitate urine (\(^{14}\)C)inulin) collection. All dogs easily tolerated this procedure without sedation or restraint.

When the dogs were studied while mildly or moderately hypovolemic, hypovolemia was achieved by a targeted removal of 15 or 30% of the calculated blood volume (approximately 12 or 24 ml/kg based on an estimated 80 ml/kg blood volume), respectively, over a period of 20 min after preparation of the animals (Adams et al., 1985). One hundred milliliters of this blood was heparinized (1000 U) and set aside for i.v. return over 10 min, from time \(t = 0\), to replace the volume of the arterial blood samples obtained over that time. The balance of the blood removed was heparinized and returned to the dog 2 h after study drug injection. No compensatory volume replacement was administered before the study to the dogs when they were made moderately or severely hypovolemic.

In the volume loading study, a volume of starch solution equal to 15% of the calculated blood volume, plus an additional 100 ml, was infused over 30 min. After the volume load, 100 ml of blood was removed, to be returned over 10 min to compensate for the rapid blood sampling.

For the normovolemia study, 100 ml of whole blood was removed from the dog, anticoagulated with 1000 U of heparin, and set aside to be reinfused during the period of frequent blood sampling. This blood was immediately replaced with 300 ml of 0.9% saline solution administered i.v. over 20 min to allow for equilibration of this volume throughout the extracellular fluid space. The study was begun not less than 30 min after volume perturbation. ICG (5 mg in 1 ml of diluent; Cardio-Green; Hynson, Westcott, and Dunning, Baltimore, MD), \(^{14}\)C]inulin (30 \(\mu\)Ci in 1.5 ml of diluent; DuPont-NEN, Boston, MA), and antipyrine (25 mg in 1 ml of diluent; Sigma Chemical Co., St. Louis, MO) were placed sequentially in a 76-cm length of i.v. tubing (4.25 ml priming volume) and connected to the proximal injection port of the PA catheter. At the onset of the study (time \(t = 0\) min), the 3.5-ml drug volume was flushed into the right atrium within 4 s using 10 ml of 5% dextrose in water, allowing the simultaneous determination of dye and thermal dilution COs. Arterial blood samples were collected every 0.05 min for the first minute and every 0.1 min for the next minute using a computer-controlled roller pump (Masterflex; Cole-Parmer, Chicago, IL), set at a withdrawal rate of 1 ml/s, and a chromatography fraction collector (model 203; Gilson, Middleton, WI). Subsequently, 30 arterial blood samples of 3 ml were drawn manually at 0.5-min intervals to 4 min, at 5 and 6 min, every 2 min to 20 min, at 25 and 30 min, every 10 min to 60 min, 15 min to 120 min, and 30 min to 360 min.

### Analytical Methods

Plasma ICG concentrations of all samples obtained up to 20 min were measured on the study day by the HPLC technique of Grasela et al. (1987) as modified in our laboratory to provide sensitivity of 0.2 to 20.00 \(\mu\)g/ml with c.v. values of 5% or less (Henthorn et al., 1992).

Plasma \(^{14}\)C]inulin concentrations of all samples were determined by liquid scintillation counting with the use of an external standard method for quench correction (Bowsher et al., 1985). Counts that were less than three times the background count were considered to be below the lower limit of detection. The c.v. of the assay was less than 3%.

Plasma antipyrine concentrations were measured in all samples using a modification of an HPLC technique developed in our laboratory (Krejcie et al., 1994, 1996a). The antipyrine method is linear from plasma concentrations of 0.10 to 10.00 \(\mu\)g/ml with c.v. values of 5% or less.

Plasma ICG and inulin concentrations were converted to blood concentrations by multiplying them by one minus the hematocrit (Hct), as neither ICG nor inulin partitions into erythrocytes. To interpret antipyrine intercompartmental clearances in relation to blood flow, plasma antipyrine concentrations were corrected for partitioning into erythrocytes by calculating the apparent dose of the respective drug assuming a red blood cell/plasma partition coefficient of 1. As the central blood flow of the antipyrine model was constrained to that of ICG and inulin (i.e., CO), the apparent antipyrine dose was estimated as a linear scalar using blood ICG and plasma antipyrine concentrations, obtained before the first evidence of recirculation according to the relationship (Krejcie et al., 1996a):

\[
\text{Apparent dose}_{\text{antipyrine}} = \frac{\text{Dose}_{\text{ICG}}}{\text{AUC}_{\text{ICG}}} \cdot \text{AUC}_{\text{antipyrine}, \text{plasma}}
\]

### Pharmacokinetic Model

The pharmacokinetic modeling methodology has been described in detail previously (Avram et al., 1997). It is based on the approach described by Jacquez (1996) for obtaining information from outflow concentration histories, the so-called inverse problem (Fig. 1). Inulin and antipyrine distributions to extracellular fluid space and total body water space, respectively, were analyzed as the convolution of their intravascular behavior, determined by the pharmacokinetics of concomitantly administered ICG, and tissue distribution kinetics (Krejcie et al., 1996a).

A delay element is a mathematical description of the frequency distribution of drug transit times that can be described by the mean transit time (MTT). The apparent volume of a delay element [e.g., central volume (VC), fast nondistributive peripheral pathway volume (VND,F); and slow nondistributive peripheral pathway volume (VND,S); Fig. 1] can be determined as the product of the flow through the delay and its MTT. The delay elements of the SAAM II kinetics analysis software (SAAM Institute, Seattle, WA) are composed of a linear chain (or tanks-in-series) of n identical compartments connected by identical rate constants \(k\) such that \(n/k\) is equal to the MTT of the delay. The solution for a linear chain obtained by

\[
\text{Fig. 1. The general model for the recirculatory pharmacokinetics of ICG, inulin, and antipyrine (Krejcie et al., 1996a). The central circulation of the three drugs receive all of CO, defined by the delay elements (VC). The delay elements are represented generically by rectangles surrounding the central circulation, CO distributes to numerous circulatory and tissue pathways that lump, on the basis of their blood volume/flow ratios or tissue volume/distribution clearance ratios (MTTs), into fast (CiND-F, VND,F) and slow (CiND-S, VND,S) peripheral blood circuits (ICG) or nondistributive peripheral pathways (inulin and antipyrine) and fast (Ci T-F, VND,S) and slow (Ci T-S, VND,S) tissue volume groups. ICG, which distributes primarily in a 76-cm length of i.v. tubing (4.25 ml priming volume) and connected to the proximal injection port of the PA catheter. At the onset of the study (time } t = 0 \text{ min), the 3.5-ml drug volume was flushed into the right atrium within 4 s using 10 ml of 5% dextrose in water, allowing the simultaneous determination of dye and thermal dilution COs. Arterial blood samples were collected every 0.05 min for the first minute and every 0.1 min for the next minute using a computer-controlled roller pump (Masterflex; Cole-Parmer, Chicago, IL), set at a withdrawal rate of 1 ml/s, and a chromatography fraction collector (model 203; Gilson, Middleton, WI). Subsequently, 30 arterial blood samples of 3 ml were drawn manually at 0.5-min intervals to 4 min, at 5 and 6 min, every 2 min to 20 min, at 25 and 30 min, every 10 min to 60 min, 15 min to 120 min, and 30 min to 360 min.

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\]
successive integration for the exit rate from compartment \( n \) is given by the Erlang frequency distribution function:

\[
f(t) = \frac{k^n \cdot t^{n-1}}{(n-1)!} \cdot e^{-kt}
\]

which is a special case (integer values for \( n \)) of the gamma distribution function (Krejcie et al., 1996b).

Arterial ICG, inulin, and antipyrine concentration-versus-time data before evidence of recirculation (i.e., first-pass data) were weighted uniformly and first fit to the sum of two right-skewed gamma distribution functions using TableCurve2D (version 3.0; Jandel Scientific, San Rafael, CA) on a Pentium-based PC (Dell Dimension, Austin, TX; Krejcie et al., 1996b). The two \( n \) values from the fit to the gamma functions were then rounded to the nearest integer value and fixed, and the data were refit to the sum of two Erlang distribution functions. Because neither ICG nor inulin distributes beyond the intravascular space before recirculation, they were modeled simultaneously to improve the confidence in the model parameters of the central (first-pass) circulation. Antipyrine has measurable tissue distribution during this time and was modeled independently; the antipyrine pulmonary tissue volume \( V_{\text{TP}} \) is the difference between the antipyrine \( V_C \) (MTPantipyrine, CO) and the central intravascular volume codetermined by ICG and inulin (MTTICG, inulin, CO). The optimum number of tanks-in-series of the peripheral delay elements, \( V_{\text{ND-F}} \) and \( V_{\text{ND-S}} \), were determined iteratively without the use of gamma or Erlang functions.

In subsequent pharmacokinetic analysis, the descriptions of the central circulation (the blood and apparent tissue volume between the right atrial injection site and the aortic bifurcation sampling site) were incorporated as parallel linear chains, or delay elements, into independent recirculatory models for the individual markers using SAAM II kinetics analysis software implemented on a Pentium-based PC (Krejcie et al., 1996b, 1997). The first-pass data were excluded from further data fitting; the results of the Erlang model of the central circulation were placed as fixed parameters into the recirculatory model, reducing the number of parameters to be optimized. The concentration-time data were weighted using the default relative reciprocal weighting of the SAAM II program. Possible systematic deviations of the observed data from the calculated values were sought (Berman et al., 1962) using the two-tailed one-sample runs test, with \( p < .05 \), corrected for multiple applications of the runs test, as the criterion for rejection of the null hypothesis (Siegel, 1956). Possible model misspecification was sought by visual comparison of the measured and predicted marker concentration-versus-time relationships.

In general, peripheral drug distribution can be lumped into identifiable volumes and clearances: a fast nondistributive peripheral pathway \( (V_{\text{ND-F}} \text{ and } C_{\text{LOD-F}}) \), a slow nondistributive peripheral pathway \( (V_{\text{ND-S}} \text{ and } C_{\text{LOD-S}}) \); rapidly (fast) equilibrating tissues \( (V_{\text{TF}} \text{ and } C_{\text{LTF}}) \); and slowly equilibrating tissues \( (V_{\text{TS}} \text{ and } C_{\text{LTS}}) \). The fast and slow nondistributive peripheral pathways (delay elements) represent intravascular circuits in the ICG and inulin models; the single nondistributive peripheral pathway in the antipyrine model, determined by the recirculation peak, represents blood flow that quickly returns the lipophilic marker to the central circulation after minimal apparent tissue distribution (i.e., it is a pharmacokinetic shunt; Krejcie et al., 1996a; Avram et al., 1997). ICG has no identifiable tissue distribution. In the inulin and antipyri ne models, the parallel rapidly and slowly equilibrating tissues are the fast and slow compartments of traditional three-compartment pharmacokinetic models, respectively; therefore, the central circulation and nondistributive peripheral pathways are detailed components of the ideal \( V_C \) of the three-compartment model (Krejcie et al., 1994). Because of the direct correspondence between the recirculatory model and three-compartment models, \( C_{\text{LO}} \) was modeled from the arterial (sampling) compartment to enable comparison of these results with previous ones.

The 8 model variables determined from arterial blood ICG concentrations, the 10 model variables determined from arterial blood antipyrine concentrations, and the 12 model variables determined from arterial blood inulin concentrations have been determined to be both sensible and identifiable for our sampling schedule by the IDENT2 program of Jacquez and Perry (1990).

Area under Blood Concentration-versus-Time Relationship (AUC). The AUC was determined for both the first-pass fit (sum of two parallel Erlang functions, AUCfirst-pass) and for the full recirculatory model. The AUCs for the full model were calculated for the interval of 0 to 3 min (AUC0–3 min). The AUCs for the full model were influenced by AUCfirst-pass, which is affected solely by changes in CO (i.e., \( \text{CO} = \text{dose/AUC}_{\text{first-pass}} \)). Therefore, the AUC resulting from recirculation of a marker was determined by subtracting the first-pass AUC from that of the full model over 3 min:

\[
\text{AUC}_{\text{recirc}} = \text{AUC}_{0–3 \text{ min}} - \text{AUC}_{\text{first-pass}}
\]

Statistical Analysis. The effects of treatment and the order of treatment on observed pharmacokinetic variables were assessed using a general linear model ANOVA for a repeated measures Latin square experimental design (NCSS 6.0.2 Statistical System for Windows; Number Cruncher Statistical Systems, Kaysville, UT). Post-hoc analysis was carried out using Fisher’s least significant difference (LSD) test. The relationships of the pharmacokinetic variables to CO were sought using standard least-squares linear regression with the Bonferroni correction of the criterion for rejection of the null hypothesis. The criterion for rejection of the null hypothesis was \( p < .05 \).

Results

Blood volume estimated by ICG total (steady-state) volume of distribution \( (V_{SS}) \) increased 20% during volume loading and decreased 9 and 22% during mild and moderate hypovolemia, respectively (Tables 1 and 2). Volume loading and both mild and moderate hypovolemia resulted in a decrease in the Hct compared with the normovolemic control (Table 1). The thermal dilution and dye (ICG) dilution COs (Tables 1 and 2, respectively) were similar \( (\text{CO}_{\text{TDD}} = 1.21 \text{ CO}_{\text{ICG}} - 1.09, r^2 = 0.91) \), indicating our sampling schedule and identification of first-pass data were appropriate; dye dilution CO increased 30% during volume loading and decreased 27 and 38% during mild and moderate hypovolemia, respectively, compared with the normovolemic control. Mean arterial pressure (MAP) decreased slightly (12%) relative to control only during moderate hypovolemia (Table 1). Neither heart rate nor systemic vascular resistance changed significantly during either volume loading or hypovolemia (Table 1).

The blood ICG, inulin, and antipyrine concentration-versus-time relationships were well characterized by the models from the moment of injection. (Figs. 2–4) The one-sample runs test confirmed that there were no systematic deviations of the observed data from the calculated values. As described later, our recirculatory model of drug disposition was able to describe the effect of altered blood volume on CO and its distribution (the ICG model, Table 2) and the effect of altered and redistributed CO on inulin (Table 3) and antipyrine (Table 4) disposition. Our model was able to account for the more than 50% increase in the area under the first minutes of the antipyrine AUC produced by moderate hypovolemia. (Fig. 4, Table 5). The extracellular fluid volume defined by the \( V_{SS} \) of inulin contracted 14 and 21% during the hypovolemic studies, mirroring changes in intravascular volume due to the transvascular fluid shift. Antipyrine \( V_{SS} \) and the
ClE values of ICG, inulin, and antipyrine were unaffected by altered blood volume (Tables 1–4).

**ICG.** The increase in total blood volume estimated by ICG VSS as a result of volume loading and the decrease during mild and moderate hypovolemia were due to changes in VC and VND-F (Fig. 1, Table 2), both of which were correlated with CO (VC = 0.06 CO + 0.58, r² = 0.64; VND-F = 0.08 CO – 0.14, r² = 0.42). Although VC increased in volume loading and decreased during hypovolemia, it always contained approximately one-third of the total blood volume. VND-F represented approximately 10% of the total blood volume in the awake normovolemic dogs; as a result of selective blood volume loss from VND-F during both mild and moderate hypovolemia, VND-F represented less than 10% of the total blood volume. The bulk of the increase in blood volume during volume loading was in VND-F, when it represented more than 20% of the estimated blood volume. No change in the volume of the slow peripheral circulations (VND-S) was observed during either volume loading or hypovolemia, as a result of which it represented less than the control 50% of estimated blood volume during volume loading and more than 50% during hypovolemia.

The increase in CO during volume loading and the decreases during mild and moderate hypovolemia were reflected almost exclusively in changes in ClND-F, which was correlated with CO (ClND-F = 0.87 CO – 2.02, r² = 0.84). ClND-F increased more than 50% during volume loading and decreased more than 50% during both mild and moderate hypovolemia. The slow intercompartmental clearance of ICG (ClND-S) and ICG ClE did not change significantly during either volume loading or hypovolemia, although ClE was correlated with CO (ClE = 0.02 CO + 0.17, r² = 0.54). The majority of the systemic distribution of CO not represented by ClE (93–96% of CO) was in ClND-F during volume loading and normovolemia but was in ClND-S during mild and moderate hypovolemia.

**Inulin.** Most of the changes in the recirculatory inulin pharmacokinetic model produced by altered intravascular
volume were in the central and fast nondistributive circuits and were, therefore, very similar to those observed for ICG (Table 3). Because $V_c$ of both ICG and inulin were modeled together, changes in $V_c$ were the same in both models. Like $V_{ND,F}$ in the ICG model, that in the inulin model was correlated with CO ($V_{ND,F} = 0.08 \text{ CO} - 0.17; r^2 = 0.80$), nearly doubling during volume loading and decreasing more than 50% during hypovolemia. $V_{ND,S}$ and the volumes of the rapidly and slowly equilibrating tissue volumes ($V_{TF,F}$ and $V_{TS,S}$, respectively), which represented more than two-thirds of the total inulin volume of distribution, were unaffected by altered intravascular volume. Changes in CO were largely reflected in inulin $Cl_{ND,F}$, which was correlated with CO ($Cl_{ND,F} = 0.85 \text{ CO} - 2.25; r^2 = 0.91$), increasing more than 50% during volume loading and decreasing more than 50% during mild and moderate hypovolemia, like that of the ICG model. Nearly 90% of CO not involved in $Cl_E$ was nondistributive in the inulin models in the dogs under all conditions because the transcapillary distribution of inulin is limited by free water diffusion. $Cl_{ND,F}$ represented the majority of the blood flow during control and volume loading studies, whereas $Cl_{ND,S}$ represented the majority of the blood flow during mild and moderate hypovolemia. Neither clearances to the rapidly and slowly equilibrating tissues ($Cl_{TF,F}$ and $Cl_{TS,S}$, respectively) nor inulin $Cl_E$ (i.e., glomerular filtration rate) changed as a result of altered intravascular volume.

**Antipyrine.** The antipyrine distribution volumes determined by the recirculatory model were modestly affected by alterations in blood volume (Table 4). The only peripheral nondistributive volume that could be independently resolved in the antipyrine model, $V_{ND}$ (Krejcie et al., 1996a), increased significantly as a result of volume loading but was unaffected by hypovolemia. Although the $V_c$ defined by ICG and inulin was increased by volume loading and decreased by hypovolemia, antipyrine $V_{TF}$ did not change. While antipyrine $V_{TF}$ did not change significantly with alterations in blood volume, it was correlated with CO ($V_{TF} = 0.80 \text{ CO} + 1.30; r^2 = 0.60$).

AUC. The antipyrine AUC for at least the first 3 min after drug administration increased more than 30% during mild hypovolemia and more than 60% during moderate hypovolemia (Fig. 4, Table 5). Because the first-pass antipyrine AUC represented approximately 50% of the AUC during the first 3 min, the increased $AUC_{0-3 \text{ min}}$ was due to not only the increase in first-pass AUCs secondary to the decreased CO but also the increase in nondistributive clearance and the decrease in distributive clearance during hypovolemia. The antipyrine AUC during the first 3 min after drug administration was only modestly (less than 20%) decreased during volume loading. Antipyrine AUC was correlated with CO both when first-pass AUC was included (e.g., $AUC_{0-3 \text{ min}} = -1.01 \text{ CO} + 16.28; r^2 = 0.60$) and when it was not (e.g., $AUC_{\text{ceci}} = -0.35 \text{ CO} + 7.23; r^2 = 0.31$). Lesser changes in the $AUC_{0-3 \text{ min}}$ and $AUC_{\text{ceci}}$ of ICG and inulin were observed during alterations in blood volume (Figs. 2 and 3, Table 5). ICG and inulin $AUC_{0-3 \text{ min}}$ values were also correlated with CO, but the correlations were not as strong as those of antipyrine (e.g., $ICG AUC_{0-3 \text{ min}} = -0.25 \text{ CO} + 6.71; r^2 = 0.42$; inulin $AUC_{0-3 \text{ min}} = -2,600 \text{ CO} + 71,500; r^2 = 0.40$).

**Discussion**

Seyde et al. (1985) studied regional blood flow redistribution in awake rats before and after hemorrhage with the use of radioactive 15-$\mu$m microspheres. They reported that blood flow from skin, muscle, gastrointestinal tract, and, to a lesser extent, kidneys was redistributed to the brain, heart, and liver in awake rats after hemorrhage of 30% of estimated blood volume resulting in a 35% decrease in CO with a 9% decrease in MAP. The relative decreases in the peripheral vascular circuits of the recirculatory pharmacokinetic models of ICG and inulin disposition during moderate canine hypovolemia (Tables 2 and 3) are consistent with these observations. ICG and inulin $Cl_{ND,F}$, which represent the non-splanchnic circulation (Krejcie et al., 1996), decreased nearly 60% in our moderately hypovolemic dogs and more than 50% in their hypovolemic rats. ICG and inulin $Cl_{ND,S}$, which represent the splanchnic circuit (Krejcie et al., 1996), decreased 20% in our moderately hypovolemic dogs and total
TABLE 3
Pharmacokinetic variables for recirculatory inulin pharmacokinetics
Values are mean (S.D.).

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Volume</th>
<th>Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$V_c^c$</td>
<td>$V_{ND-F}^c$</td>
</tr>
<tr>
<td>Volume loaded</td>
<td>1.10</td>
<td>0.59$^d$</td>
</tr>
<tr>
<td>Normovolemic</td>
<td>1.03</td>
<td>0.32</td>
</tr>
<tr>
<td>Mildly hypovolemic</td>
<td>0.86$^{d,e}$</td>
<td>0.15$^{d,e}$</td>
</tr>
<tr>
<td>Moderately hypovolemic</td>
<td>0.71$^{d,e,f}$</td>
<td>0.13$^{d,e}$</td>
</tr>
</tbody>
</table>

a The volumes (V) of the central (C), rapidly equilibrating (fast) nondistributive (ND-F), and slowly equilibrating nondistributive (ND-S) circuits and the rapidly equilibrating (fast) (T-F) and slowly equilibrating (T-S) tissues, and $V_{SS}$, which equals the sum of all volumes.
b The clearances (Cl) of the rapidly equilibrating (fast) nondistributive (ND-F) and slowly equilibrating nondistributive (ND-S) circuits and the rapidly equilibrating (fast) (T-F) and slowly equilibrating (T-S) tissues, $Cl_E$, and $Cl_{ND-F}$, $Cl_{ND-S}$, and $Cl_{T-F}$, $Cl_{T-S}$, which equals the ICG (dye dilution) CO determined at the moment of marker injection.
c Correlated with CO ($p < .05$).
d Significantly different from volume loaded ($p < .05$), as determined by Fisher’s LSD test.
e Significantly different from normovolemic control ($p < .05$), as determined by Fisher’s LSD test.
f Significantly different from mildly hypovolemic ($p < .05$), as determined by Fisher’s LSD test.

TABLE 4
Pharmacokinetic variables for recirculatory antipyrine pharmacokinetics
Values are mean (S.D.).

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Volume</th>
<th>Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$V_c^c$</td>
<td>$V_{ND}^c$</td>
</tr>
<tr>
<td>Volume loaded</td>
<td>1.10</td>
<td>0.09</td>
</tr>
<tr>
<td>Normovolemic</td>
<td>1.03</td>
<td>0.08</td>
</tr>
<tr>
<td>Mildly hypovolemic</td>
<td>0.86$^{d,e}$</td>
<td>0.09</td>
</tr>
<tr>
<td>Moderately hypovolemic</td>
<td>0.71$^{d,e,f}$</td>
<td>0.11</td>
</tr>
</tbody>
</table>

a The volumes (V) of the central (C) rapidly equilibrating (fast) nondistributive (ND-F), pulmonary tissue (the difference between the antipyrine central circuit volume and that described by ICG and inulin) (T-P), nondistributive (ND) circuit, and the rapidly equilibrating (fast) (T-F) and slowly equilibrating (T-S) tissues, and $V_{SS}$, which equals the sum of all volumes.
b The clearances (Cl) of the nondistributive (ND) circuit and the rapidly equilibrating (fast) (T-F) and slowly equilibrating (T-S) tissues, elimination clearance ($Cl_{E}$), and $Cl_{ND}$, which equals the ICG (dye dilution) CO determined at the moment of marker injection.
c Correlated with CO ($p < .05$).
d Significantly different from normovolemic control ($p < .05$), as determined by Fisher’s LSD test.
e Significantly different from volume loaded ($p < .05$), as determined by Fisher’s LSD test.
f Significantly different from mildly hypovolemic ($p < .05$), as determined by Fisher’s LSD test.

TABLE 5
Areas under the blood concentrations of the physiological markers versus time relationships (AUC0–3 min) and those due to recirculation alone (AUCrecirc) for the first 3 min after right atrial injection of ICG, inulin, and antipyrine
Values are mean (S.D.).

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>ICG</th>
<th>Inulin</th>
<th>Antipyrine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC0–3 min$^a$</td>
<td>AUCrecirc$^a$</td>
<td>AUC0–3 min$^a$</td>
</tr>
<tr>
<td>Volume loaded</td>
<td>4.16</td>
<td>3.53</td>
<td>4.61</td>
</tr>
<tr>
<td>Normovolemic</td>
<td>4.93</td>
<td>4.09</td>
<td>4.92</td>
</tr>
<tr>
<td>Mildly hypovolemic</td>
<td>5.43</td>
<td>4.26</td>
<td>5.84</td>
</tr>
<tr>
<td>Moderately hypovolemic</td>
<td>6.47$^{b,c}$</td>
<td>5.00$^{b,c}$</td>
<td>6.98$^{b,c}$</td>
</tr>
</tbody>
</table>

a Correlated with CO ($p < .05$).
b Significantly different from normovolemic control ($p < .05$) as determined by Fisher’s LSD test.
c Significantly different from volume loaded ($p < .05$), as determined by Fisher’s LSD test.
hepatic flow decreased 19% in their hypovolemic rats. ICG hepatic Cl\textsubscript{g} did not change as a result of hypovolemia because it is not hepatic blood flow dependent in the dog, whereas insulin Cl\textsubscript{g} did not change as a result of hypovolemia because canine renal blood flow does not change during nonhypotensive hemorrhage (Vatner, 1974).

To identify a pharmacokinetic basis of differences in reactivity to drugs with rapid onsets of effect, drug distribution kinetics during the time of expected onset and offset of effect must be described in detail. This requires a model that accounts for the role of CO and its distribution on drug disposition. The present study found significant changes in antipyrine disposition in dogs during mild and moderate hypovolemia using a recirculatory pharmacokinetic model based on frequent early arterial blood samples (Table 4). Another study of drug pharmacokinetics during canine hypovolemia (Adams et al., 1985) failed to find an explanation for increased reactivity to drugs with a rapid onset of central nervous system effects during hypovolemia. However, their study described midazolam disposition using infrequent blood samples obtained beginning at 15 min, which is long after the drug has produced its maximal effect. As a result of their sampling schedule, they were only able to characterize late drug distribution and global pharmacokinetic parameters, which are unlikely to explain differences in reactivity to drugs with a rapid onset of effect.

An important observation of the present application of the recirculatory pharmacokinetic model is that mild hypovolemia increased antipyrine AUC by approximately one-third in the critical first minutes after drug administration, whereas moderate hypovolemia increased antipyrine AUC by more than 50% (Table 5). Increased AUC during mild and moderate hypovolemia was due to maintenance of the apparent first minutes after drug administration. AUC is often used as a measure of drug exposure (Powis, 1985); increased arterial drug concentrations resulting from a larger nondistributive clearance increase drug exposure of the sites of action of lipophilic drugs with a rapid onset of effect, for which antipyrine is a pharmacokinetic prototype, and would be expected to result in a more profound and prolonged effect of these drugs, as predicted by Price (1960). Most of this increase in AUC is due to an increased return of drug to the central circulation and not to a first-pass increase resulting from decreased CO.

Despite increased fast tissue distributive flow with increased CO in volume-loaded animals, the less than 20% decrease in antipyrine AUC for the first minutes after drug administration (Table 5) was not as profound as might be expected based on results observed in hypovolemic dogs because nondistributive flow also increased during volume loading (Table 4). Nevertheless, one would expect a modest increase in the dose requirements for rapidly acting drugs in volume loaded subjects based on the decrease in AUC.

The majority of CO in the recirculatory insulin model is nondistributive (Table 3). Thus, although insulin nondistributive clearance, especially Cl\textsubscript{ND-F}, changed significantly with CO in animals with altered blood volume, insulin AUC increases during hypovolemia and its decrease during volume loading were approximately half those observed for antipyrine (Table 5). As a result, the onset and duration of effect of the hydrophilic drugs for which insulin is a prototype (e.g., neuromuscular blockers) will not be as profoundly increased in hypovolemia and decreased in volume loading as those of the lipophilic drugs for which antipyrine is the prototype.

Although the decrease in CO during hypovolemia did not affect antipyrine Cl\textsubscript{ND}, it decreased Cl\textsubscript{T-F} by 42% and Cl\textsubscript{T-S} by 56% (Table 4). The rapidly equilibrating (fast) tissue volume represents splanchnic tissues, whereas the slowly equilibrating tissue volume represents nonsplanchnic tissues (Sedek et al., 1989), to which distributive blood flows were proportional to the blood flows of the fast intravascular circuit of the ICG model (Cl\textsubscript{T-F} = 0.65 ICG Cl\textsubscript{ND-F} + 1.63, r\textsuperscript{2} = 0.68; Cl\textsubscript{T-S} = 0.21 ICG Cl\textsubscript{ND,F} + 0.69, r\textsuperscript{2} = 0.52). The decreased intercompartmental clearance of urea (like antipyrine, a marker of total body water) during dialysis that we observed in an earlier study (Bowsher et al., 1985) may reflect the decrease in blood volume observed in the absence of significant hypotension during hemodialysis (Mann et al., 1989). Antipyrine hepatic Cl\textsubscript{g} did not change during hypovolemia because it is not blood flow limited.

Hemodynamic responses to acute hypovolemia in conscious mammals have two well-defined phases: a sympathoexcitatory phase and a sympathoinhibitory phase (Schadt and Ludbrook, 1991). As a result of sympathetic vasoconstriction, acute blood losses of less than 25 to 30% of blood volume are characterized by little or no decrease in MAP despite a decrease in CO. General vasodilation (except in skin) at more extreme acute blood losses of less than 25% to 30% of blood volume are characterized by little or no decrease in MAP despite a decrease in CO. General vasodilation (except in skin) at more extreme acute blood losses results in a precipitous fall in MAP with a continued decrease in CO. The blood volumes removed in the present mild and moderate hypovolemic studies were designed to be less than those required to precipitate the sympathoinhibitory phase of blood loss because we intended to perform repeated studies in the same animals and extreme blood loss is associated with potential organ damage and death. The 22% decrease in blood volume in moderately hypovolemic animals of the present study produced only a 12% decrease in MAP despite a 38% decrease in CO, suggesting successful avoidance of the sympathoinhibitory response to blood loss (Table 1). The effects of more extreme blood loss cannot be extrapolated from these results, given the significantly different physiology accompanying more extreme acute blood loss.

The studies were conducted in splenectomized dogs because the canine spleen is capable of autotransfusing 5 to 6
ml of erythrocytes/kg of body weight during hemorrhage (Hoeckstra et al., 1988). The blood volume reductions in the present hypovolemia studies were less than those targeted because the blood volume removed was based on low initial blood volume estimates from our previous studies in nonsplenectomized dogs and because transvascular fluid shifts replace 20 to 35% of the bled volume rapidly and reduce the extravascular fluid volume (inulin $V_{ss}$; Hinghofer-Szalkay, 1986). The dilutional effects of the transvascular fluid shifts account for the reduced Hct in the hypovolemia studies, whereas the dilutional and osmotic effects of the starch solution explain the reduced Hct in the volume-loading studies.

Because anesthesia not only affects blood flow and drug distribution (Avram et al., 1997) but also attenuates or eliminates the vasoconstriction characteristic of the sympathoexcitatory phase of hemorrhage (Schadt and Lubrook, 1991), the present study was conducted in awake animals. Changes in blood flow distribution accompanying hypovolemia are further complicated by the changes caused by potent volatile anesthetics (Seyde and Longnecker, 1984) and i.v. anesthetics (Seyde et al., 1985), with unknown consequences for drug disposition.

Hypovolemia caused an increase in antipyrine AUC for the first minutes after drug administration due to the increased fraction of CO represented by nondistributive blood flow during hypovolemia. For rapidly acting drugs, such as the centrally acting i.v. anesthetics for which the lipophilic marker antipyrine is a prototype, maintenance of nondistributive blood flow despite a decrease in CO would produce a more profound and longer-lasting drug effect due to exposure of potential sites of drug action to higher drug concentrations for a longer period of time. This provides a rationale for altered drug dosing in patients with altered blood volume.

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