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Red Blood Cells: A Neglected Compartment in Pharmacokinetics and Pharmacodynamics

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I. Introduction

The significance of studying the kinetics of drug partitioning into red blood cells (RBCs[b]) in animals and humans is not fully appreciated, although the importance of routine determination of rate and extent of partitioning of investigational drugs has been stressed (Lee et al., 1981b; Hinderling 1984). As will be demonstrated in this review, failure to determine the kinetics of drugs in RBCs may be a lost opportunity. Knowledge of RBC partitioning of compounds enables: (*a*) a rational choice of appropriate biological fluid, either whole blood, plasma, or serum, for assay; (*b*) physiologically meaningful referencing of pharmacokinetic parameters of drugs to concentrations in either whole blood, plasma, or serum; (*c*) in vitro prediction of drug distribution in vivo; (*d*) determination of plasma protein binding of drugs; and (*e*) effective screening of drugs whose biophase resides within the RBCs, thereby enabling the study of the effects of drugs on RBCs.

The goals of this review are to: (*a*) summarize the present knowledge regarding the partitioning of drugs into RBCs, and (*b*) demonstrate the relevance of knowing the kinetics of RBC uptake of drugs. RBCs of animals and humans are known to be different (Bowyer, 1957). This review deals almost exclusively with human RBCs and their interactions with drugs.

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[b] Abbreviations: **AED**, equilibrium dialysis through artificial semipermeable membrane; **BED**, biological equilibrium dialysis through biological semipermeable membrane; **Ce**, drug concentrations in the RBCs; **Clb**, clearance of drug referenced to the drug concentration in whole blood; **Clp**, clearance of drug referenced to the drug concentration in plasma; **Cp**, drug concentrations in plasma; **fu**, fraction of drug in plasma unbound; **Hc hematocrit**, relative volume of RBCs in whole blood, RBCs suspended in plasma/serum, or plasma water/buffer; **I**, constant relating the unbound drug concentration in the aqueous phase of the RBCs to the unbound drug concentration in plasma or serum; **Kb/p**, whole blood to plasma partition coefficient of drug obtained from the ratio of the concentrations in whole blood and plasma or serum; **Ke/p**, RBC-to-plasma partition coefficient of drug obtained from the ratio of the concentrations in RBCs and plasma or serum; **Ke/p,u**, RBC-to-plasma water partition coefficient of drug obtained from the ratio of the concentrations in RBCs and plasma water or buffer; **nEt**, total binding site concentration in the RBCs; **nPt**, total binding site concentration in plasma or serum; **RBC**, red blood cell; **Vu,ss**, steady-state volume of distribution referenced to the unbound drug concentration in plasma water determined in vivo.

II. Salient Features of Red Blood Cells

Among the cellular constituents of blood, the RBCs represent, by far, the largest population both in number and cell size. The RBCs make up more than 99% of the total cellular space of blood in humans (Diem and Lentner, 1975c). RBCs occupy a volume of approximately 25 to 30 $mL \cdot kg^{-1}$, of which 71% constitute an aqueous phase (Diem and Lentner, 1975a). A total of approximately 760 g of hemoglobin is contained in the RBCs, representing approximately 10% of the total body proteins of an adult human (Spector, 1956; Diem and Lentner, 1975c; Kawai et al., 1994). Hemoglobin interacts with small diffusible ligands such as O_2, CO_2, and NO and may be involved in the control of blood pressure (Jia et al., 1996). The RBCs draw energy from glucose metabolism via direct glycolysis and the hexose monophosphate shunt (Beutler et al., 1995a). Individual RBCs are biconcave discs and have a cell diameter of 7 to 9 μm and a thickness of 2 μm (Diem and Lentner, 1975a; Beutler et al., 1995b). The volume of individual RBCs is 90 fL and the surface area is 163 μm^2 (Diem and Lentner, 1975b; Beutler et al., 1995a). The plasma membrane consists of a lipid bilayer of 10-nm width and contains channels of 0.4-nm radius (Solomon et al., 1983; Delaunay, 1995). The membrane potential is inside negative (Hoffman and Laris, 1974). The main constituents of the membrane are phospholipids, cholesterol, and proteins (Beutler et al., 1995b). Linked to the inner surface of the plasma membrane is a supramolecular system of skeleton proteins (Delaunay, 1995). The plasma membrane proteins include receptors, carriers, and enzymes (Gratzer, 1981; Benjamin and Dunham, 1983; Carruthers and Melchior, 1988). The carriers include anion exchanger (Cl^-/HCO_3^-), glucose transporter, cation transporters (Na^+/K^+ pump, Ca^{2+} pump, Ca^{2+} activated K^+ channel, Na^+/H^+ exchanger, and Na^+/Li^+ cotransporter), anion/cation cotransporters ($Na^+/K^+/2Cl^-$ cotransporter and K^+/Cl^- cotransporter), nucleoside transporter, and others (Belz et al., 1972; Benjamin and Dunham, 1983; Carruthers and Melchior, 1988; Lauf et al., 1992; Van Belle, 1993; Garay et al., 1994; Delaunay, 1995). Additional enzymes and drug-binding proteins are located in the cytosol (Agarwal et al., 1986; Cossum, 1988; Hooks, 1994). The various enzymes in the RBCs can metabolize many drugs and an excellent review of this topic was presented by Cossum in 1988. An updated list of drugs metabolized by RBCs is presented in table 1. The cytosolic pH of the RBCs is 7.1 to 7.3 (Funder and Wieth, 1966) and smaller than the pH of plasma water (7.4), which is a consequence of the partitioning of electrolytic drugs. Drugs may bind to the membrane and/or to hemoglobin, carboanhydrase, and binding proteins in the cytosol of the RBCs (Kwant and Seeman, 1969; Wind et al., 1973; Beerman et al., 1975; Bickel, 1975; Wallace and Riegelman, 1977; Ehrnebo, 1980).

The RBCs belong to the cellular space within the body and share many characteristics with other cells of the body. However, RBCs differ from other body cells and blood cells such as leukocytes in that they lack a nucleus. They are devoid of endoplasmic reticulum, cytochrome P450 isozymes, and mitochondria (Cossum, 1988). Also, individual RBCs are unlike tissue cells in that they are not connected with each other but are suspended in blood plasma. RBCs have a life span of 100 to 120 days, during which they travel 250 km throughout the cardiovascular system (Beutler et al., 1995a). RBCs are easily accessible and can be kept functional for prolonged times under in vitro conditions.

Investigations of the RBC partitioning of relatively small organic cationic, anionic, and nonelectrolytic molecules have shown that lipophilicity, molecular size, and chiral characteristics are important (Giebel and Passow, 1960; Sha'afi et al., 1971; Deuticke, 1977). Lipophilic organic compounds penetrate the RBCs by dissolving into the lipid bilayer membrane (Schanker et al., 1961, 1964; Holder and Heyes, 1965). Small size hydrophilic compounds (<150 d) enter the RBCs through aqueous channels. RBC partitioning by passive diffusion has been reported for organic cationic and anionic drugs, as well as for nonelectrolytes (Schanker et al., 1961, 1964; Holder and Hayes, 1965; Hinderling, 1984; Shirkey et al., 1985; Sweeney et al., 1988; Ferrari and Cutler, 1990; Lin et al., 1992; Reichel et al., 1994).

In theory, rate and extent of RBC partitioning of drugs can be determined ex vivo or in vitro. In the ex vivo procedure, drug is administered to humans, a series of blood samples is taken, and, following centrifugal separation, the drug concentrations are measured in the RBCs and plasma. In the in vitro procedure, drug is added to RBCs suspended in plasma or in plasma water, and after mixing, the drug's concentration is measured in RBCs and plasma or plasma water following centrifugal separation (fig. 1; Hinderling, 1984). Apart from practical considerations that favor the latter method over the former, there exist additional reasons to prefer the in vitro procedure. With many drugs, the rate of partitioning is fast, and distribution equilibrium is reached within a few seconds to minutes; in these cases, only the in vitro procedure enables determination of the rate of partitioning to obtain meaningful results. The extent of drug partitioning into RBCs should be determined under steady-state equilibrium conditions. Because the in vitro method uses a closed system, steady-state equilibrium conditions can be easily established. With rapidly penetrating drugs, quasi steady state of equilibrium conditions may also be reached under in vivo conditions even though the human body represents an open system. However, with more slowly equilibrating drugs, this is not the case, and the erythrocyte uptake process is more difficult to separate from the multiplicity of other kinetic events, such as tissue distribution and elimination from the body, which are occurring simultaneously. This is not an issue with the in vitro procedure (Wallace and Riegelman, 1977).

TABLE 1
Drugs metabolized by blood cells in humans

Compound	Reference
Acetylsalicylic acid[b]	Harris and Riegelman (1967)
	Rylance et al. (1981)
	Costello et al. (1984)
N-Acetylcystein[b]	Keith et al. (1984)
4-Aminophenol[b]	Eckert (1988)
Azathioprine[b]	Chalmers et al. (1967)
	Ellion and Hitchings (1975)
Bunolol[b]	Leinweber and DiCarlo (1974)
Captopril[b]	Keith et al. (1984)
Chlorpromazine[b]	Traficante et al. (1979)
Dapsone[b,c]	Drayer et al. (1974)
Daunorubicin[b,c]	Huffman and Bachur (1972)
Dehydroepiandrosterone[b]	Morsches et al. (1981)
Didanosin[b]	Back et al. (1992)
	Barry et al. (1993)
Dopamine[b]	Männl and Hempel (1972)
	Ratge et al. (1991)
Epinephrine[b]	Männl and Hempel (1972)
	Dannon and Sapira (1972)
Esmolol[b]	Quon and Stampfli (1985)
	Gorczynski (1985)
Estradiol[b], Estrone[b]	Repke and Markwardt (1954)
	Migeon et al. (1962)
	Jacobsohn et al. (1975)
Etoposide[b]	Loo et al. (1987)
5-Fluorouracil[a]	Schaaf et al. (1986)
Haloperidol[b]	Inaba et al. (1989)
	Chan et al. (1992)
Heroin[b]	Owen and Nakatsu (1983)
Insulin[b]	Gambhir et al. (1981)
	Nerurkar and Gambhir (1981)
Isoproterenol[b], isosorbide dinitrate[b,d]	Männl and Hempel (1972)
	Bennett et al. (1983)
	Bennett et al. (1985)
LY 217896[b]	Bonate and Peyton (1995)
6-mercaptopurine[b]	Weinshilboum and Sladek (1980)
	Pazmiño et al. (1980)
	Rostami-Hodjegan et al. (1995)
Misonidazole[b]	Loo et al. (1987)
Nitroglycerin[a,b], metabolites[a,b]	Armstrong et al. (1980)
	Noonan and Benet (1982)
	Sokoloski et al. (1983)
	Bennett et al. (1985)
	Cossum and Roberts (1985)
	Chong and Fung (1989)
Norepinephrine[b]	Axelrod and Cohn (1971)
	Männl and Hempel (1972)
	Ratge et al. (1991)
Para-aminobenzoic acid[b,c]	Blondheim (1955)
	Motulski and Steinmann (1962)
	Mandelbaum-Shavit and Blondheim (1981)
Para-aminosalicylic acid[b,c]	Motulski and Steinmann (1962)
	Drayer et al. (1974)
Penicillamin[b]	Keith et al. (1985)
Pentaerythritol tetranitrate[b]	DiCarlo et al. (1965)
Pentoxyphillin[a]	Bryce et al. (1980)
	Ings et al. (1992)
Procainamide[a]	Drayer et al. (1974)
	Chen et al. (1983)
Procaine[a,c]	Calvo et al. (1980)
Progesterone[b]	Van der Molen and Groen (1968)
Ribavirin[b]	Zimmerman and Deeprose (1978)
	Page and Connor (1990)
Sulfanilamide[b,c]	Blondheim (1955)
Testosterone[b]	Van der Molen and Groen (1968)
	Mulder et al. (1972)
Thioguanine[b]	Lennard and Maddocks (1983)
Thiospirolactone[b]	Keith et al. (1984)

[a] Blood cells.
[b] Red blood cells.
[c] White blood cells.
[d] Gender-related difference in rate of metabolism.

To date, only few data that allow a comparison of the results obtained by the in vitro and ex vivo methods have been reported (Fleuren and Van Rossum, 1977; Veronese et al., 1980; Kawai et al., 1982; Borgå and Lindberg, 1984; Brocks et al., 1984; Hinderling, 1984; Shirkey et al., 1985). The extent of RBC partitioning using the two procedures was reportedly similar for digoxin, terbutaline, amiodarone, and chlorthalidone (Fleuren and Van Rossum, 1977; Veronese et al., 1980; Borgå and Lindberg, 1984; Hinderling, 1984). However, discrepancies between the two methods were apparent for the estimated rate of partitioning for terbutaline and digoxin, as well as for the extent of partitioning for hydroxychloroquine (Kawai et al., 1982; Borgå and Lindberg, 1984; Brocks et al., 1984). With terbutaline, the in vitro experiment was conducted at room temperature (Borgå and Lindberg, 1984), and rates of drug partitioning into RBCs are known to be temperature-dependent in many cases (Hinderling, 1984; Reichel et al., 1994). There is clearly a need for more investigations comparing the results on the RBC partitioning of drugs measured under appropriate in vitro and ex vivo conditions. The influence of the composition of the suspension fluid and the impact of repeated washing of the RBCs on the results obtained by the in vitro method should also be carefully delineated.

III. Principles and Definitions

The in vitro method for determining extent and rate of RBC partitioning of drugs uses red cell suspensions in plasma or plasma water. Alternatively, serum instead of plasma or a pH 7.4 buffer instead of plasma water may be used. The experiments are conducted at pH 7.4 and 37°C. The rate of drug partitioning into RBCs is determined in spiked whole blood or in a suspension of RBCs in plasma water, which are gently shaken to mimic the in vivo situation, where drug distribution occurs by diffusion and convection. Timed samples are taken, which are immediately cooled and centrifuged (Hinderling, 1984; Reichel et al., 1994). Subsequently, the drug concentrations in the separated RBCs and plasma (or plasma water) are determined, and the times required to reach equilibration between drug concentrations in the RBCs and plasma (or plasma water) are calculated. The

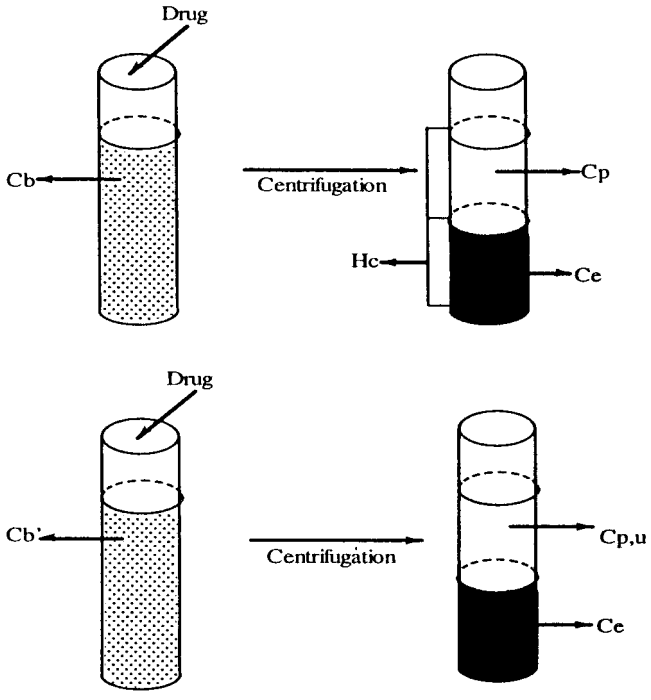

FIG. 1. Determination of rate and extent of red blood cell partitioning of drugs in whole blood or in red blood cells suspended in plasma or buffer. Cb, drug concentration in whole blood; Cb′, drug concentration in red blood cell suspension (red blood cells suspended in plasma water or buffer); Cp, drug concentration in plasma; Cp,u, unbound drug concentration in plasma water or buffer; Ce, drug concentration in red blood cells; Hc, hematocrit.

extent of RBC partitioning of drugs, K, is obtained by measuring, after equilibration and subsequent centrifugal separation, the respective drug concentrations in the RBCs (Ce) and in plasma (Cp), or in plasma water (Cp,u), in accordance with equations (1) and (2) (fig. 1):

$$Ke/p = Ce/Cp \quad [1]$$

$$Ke/p,u = Ce/Cp,u \quad [2]$$

The relationship between Ke/p and Ke/p,u is given by:

$$Ke/p = Ke/p,u \cdot fu \quad [3]$$

where fu represents the fraction of drug unbound in plasma. Equation (3) indicates that Ke/p depends on fu. This is because only unbound drug molecules in plasma partition into RBCs (Kurata and Wilkinson, 1974; Hinderling, 1984).

The whole blood-to-plasma concentration ratio (Kb/p) represents an additional drug distribution parameter of interest. The relationship between Ke/p and Kb/p is defined by:

$$Kb/p = Ke/p \cdot Hc + (1 - Hc) \quad [4]$$

Equation (4) indicates that Kb/p depends on the hematocrit (Hc) of the whole blood used in the determination. In contrast to Kb/p, both Ke/p and Ke/p,u are independent of Hc. The RBC partitioning of drugs has also been defined in terms of ratios of amounts in RBCs to those in plasma or plasma water (Ehrnebo and Odar-Cederlöf, 1975), but the partition coefficients so computed are dependent on Hc, which makes the comparison of the RBC partitioning across studies and drugs difficult. It follows from equation (4) that (*a*) Kb/p = Ke/p if Ke/p = 1, (*b*) Kb/p > Ke/p if Ke/p < 1, and (*c*) Kb/p < Ke/p if Ke/p > 1.

Ke/p,u can be interpreted as a measure of the absolute affinity of drug to the binding sites in the RBCs, whereas Ke/p expresses a drug's affinity to binding sites in the RBCs, relative to those in plasma (e.g., those associated with albumin and α_1-acid glycoprotein). After addition of drug to suspensions of RBCs in plasma, the binding sites in plasma and in the RBCs compete for drug.

For drugs with saturable plasma protein binding, Ke/p increases with increasing whole blood concentration, even though Ke/p,u may be concentration-independent. Constancy of the RBC partitioning over a defined drug concentration range can be verified by using a system of RBCs suspended in plasma water or buffer.

A nonelectrolyte drug that is distributed exclusively into the aqueous phase of the RBCs (71% of total RBC volume) is expected to have a value of Ke/p,u = 0.71. A value of Ke/p,u > 0.71 for a nonelectrolyte drug indicates additional binding to erythrocytic binding sites. For cationic and anionic drugs, the respective critical Kp/e,u values indicative for erythrocytic drug binding were postulated to be different as a result of the Donnan equilibrium (Schanker et al., 1961, 1964).

Drugs may bind to constituents in the RBCs and plasma. It is assumed that one class of drug binding sites exists in both RBCs and plasma. The concentration in the RBCs for drugs lipophilic enough to pass the RBC membrane is given by:

$$Ce = Cp,u\left[I + \frac{nEt/K_{D,e}}{1 + Cp,u/K_{D,e}}\right] \quad [5]$$

where nEt corresponds to the total binding site concentration in the RBCs, $K_{D,e}$ represents the association constant and I is a constant relating the unbound drug concentration in the aqueous phase of the RBCs and Cp,u.

For hydrophilic drugs that only bind to the outer part of the membrane without actually passing it, Ce is defined by equation (6):

$$Ce = Cp,u\left[\frac{nEt/K_{D,e}}{1 + Cp,u/K_{D,e}}\right] \quad [6]$$

The total drug concentration in plasma is defined by:

$$Cp = Cp,u\left[1 + \frac{nPt/K_{D,p}}{1 + Cp,u/K_{D,p}}\right] \quad [7]$$

where nPt and $K_{D,p}$ correspond to the total binding site concentration in plasma and the association constant, respectively.

Combination of equations (5) and (7) yields the following expression for Ke/p of minimally lipophilic compounds:

$$Ke/p = \left[I + \frac{nEt/K_{D,e}}{1 + Cp,u/K_{D,e}}\right] \Big/ \left[1 + \frac{nPt/K_{D,p}}{1 + Cp,u/K_{D,p}}\right] \quad [8]$$

The corresponding equation for hydrophilic compounds is:

$$Ke/p = \left[\frac{nEt/K_{D,e}}{1 + Cp,u/K_{D,e}}\right] \Big/ \left[1 + \frac{nPt/K_{D,p}}{1 + Cp,u/K_{D,p}}\right] \quad [9]$$

Equations (8) and (9) are nonlinear and predict that if Cp,u exceeds a critical value, binding of drug to the sites in the RBCs and/or in plasma becomes saturable. Hence, Ke/p will not be constant and either decreases or increases with increasing Cp,u.

Special cases for sufficiently lipophilic compounds can be differentiated as follows: (*a*) both binding sites in RBCs and in plasma are saturated:
$Cp,u \gg K_{D,e}$ and $Cp,u \gg K_{D,p}$:

$$Ke/p = \frac{I + nEt/Cp,u}{1 + nPt/Cp,u} \rightarrow I \quad \text{for} \quad Cp,u \rightarrow \infty \quad [10]$$

(*b*) both binding sites in RBCs and plasma are unsaturated: $Cp,u \ll K_{D,e}$ and $Cp,u \ll K_{D,p}$:

$$Ke/p = \frac{I + nEt/K_{D,e}}{1 + nPt/K_{D,p}} = \text{constant} \quad [11]$$

(*c*) only binding sites in RBCs are saturated: $Cp,u \gg K_{D,e}$ and $Cp,u \ll K_{D,p}$:

$$Ke/p = \frac{I + nEt/Cp,u}{1 + nPt/K_{D,p}} \rightarrow \frac{I}{1 + nPt/K_{D,p}} \quad \text{for} \quad Cp,u \rightarrow \infty \quad [12]$$

or (*d*) only binding sites in plasma are saturated:

$Cp,u \ll K_{D,e}$ and $Cp,u \gg K_{D,p}$:

$$Ke/p = \frac{I + nEt/K_{D,e}}{1 + nPt/Cp,u} \rightarrow I + nEt/K_{D,e} \quad \text{for} \quad Cp,u \rightarrow \infty \quad [13]$$

IV. State of the Art

A. Methodological Aspects

Meaningful in vitro determinations of rate and extent of RBC partitioning of drugs must be performed under controlled physiological conditions (pH = 7.4; temperature = 37°C) and over the entire clinically relevant concentration range of drug. It is prudent to study type (linear or nonlinear) and reversibility of the RBC partitioning of drugs. With racemic drugs, the possible stereospecificity of the RBC uptake kinetics of the individual enantiomers should be investigated. True and apparent stereospecific RBC uptake of racemates must be differentiated. This requires determination of Ke/p and Ke/p,u for the individual enantiomers. Different Ke/p,u values for the enantiomers indicate true stereospecific RBC uptake. Identical Ke/p,u values and different Ke/p values suggest stereospecific plasma protein binding. Reversibility of drug binding to the RBCs can be studied in repartitioning experiments, in which drug-loaded RBCs are resuspended in drug-free plasma or plasma water. If the kinetics of drug in partitioning and repartitioning experiments are found to be identical, irreversible drug binding to RBC constituents can be excluded (Hinderling, 1984). It is prudent to first acquire knowledge on the rate of partitioning of drug to estimate when steady state of equilibrium is reached so that the extent of RBC partitioning can be appropriately estimated.

As mentioned above, RBCs contain numerous enzymes, which under in vitro and ex vivo conditions have been shown to metabolize drugs contained in whole blood. It is therefore prudent to use specific assay methodologies separating parent drug and possible metabolite concentrations in determining rate and extent of RBC partitioning of compounds.

With some drugs, uptake in white blood cells and platelets is also significant (Hebden et al., 1970; Piazza et al., 1981; Bergqvist and Domeij-Nyberg, 1983; Sartoris et al., 1984). When aliquots of whole blood from subjects taking chloroquine were collected into tubes that either did or did not contain anticoagulant, and subsequently were centrifuged, the drug concentrations in serum were 4 times larger than in plasma (Bergqvist and Domeij-Nyberg, 1983). The larger concentrations in serum were explained by a release of drug from the platelets during blood coagulation. Similar results were obtained with desmethylchloroquine (Bergqvist and Domeij-Nyberg, 1983). Therefore, the widely held assumption that serum and plasma concentrations of drug are identical may not be correct for some drugs.

Spuriously elevated Ke/p and Kb/p values for cationic drugs bound to α_1-acid glycoprotein are obtained if blood collection tubes with stoppers containing tris(2-butoxyethyl)phosphate, a plasticizer, are used (Fremstad and Bergerud, 1976; Midha et al., 1979). Occurrence of hemolysis will also affect the results of RBC partitioning experiments and must be avoided.

B. Rate of Partitioning

Most of the studies investigating the RBC partitioning of drugs have used blood from healthy volunteers. Fewer investigations employed blood from patients. The kinetics of partitioning into RBCs, i.e., cytokinetics, have been systematically investigated in vitro only with relatively few drugs (Wallace and Riegelman, 1977; Hinderling, 1984; Lin et al., 1992; Minami and Cutler, 1992; Reichel et al., 1994). In some cases, evidence was

obtained, suggesting that passive diffusion through the RBC membrane was the underlying mechanism (Schanker et al., 1961; Hinderling, 1984; Sweeney et al., 1988; Shirkey et al., 1985). For salicylic acid and other hydroxybenzoic acids, parallel transport by both the anion exchanger and passive diffusion was postulated (Minami and Cutler, 1992). However, for chloroquine, contradictory results indicative of passive diffusion or carrier-mediated transport were reported (Yayon and Ginsburg, 1982; Ferrari and Cutler, 1990). With passive diffusion, the driving force is the unbound drug concentration in plasma water. For drugs with sufficient lipophilicity to pass the RBC membrane, equilibration is reached when the ratio of the unbound concentrations of drug in the aqueous phases of plasma and RBC cytosol remains constant. Within the RBCs, different kinetic subcompartments have been identified with digoxin and derivatives, and a compartment-model–independent parameter such as the mean transit time has been proposed as a measure for the rate of drug partitioning into the RBCs (Hinderling, 1984). Large differences in the rate of RBC partitioning between structurally related and unrelated compounds have been observed (Schanker et al., 1961, 1964; Kornguth and Kunin, 1976; Wallace and Riegelman, 1977; Skalski et al., 1978; Jun and Lee, 1980; Hinderling, 1984; Matsumoto et al., 1989; Ferrari and Cutler, 1990; Reichel et al., 1994). The estimated time to reach partitioning equilibrium between RBCs and plasma or plasma water ranges between a few seconds to several hours for different drugs (Hinderling, 1984; Reichel et al., 1994).

Several drugs with primary amino groups such as gentamycin, furosemide, procainamide, bumetanide, methotrexate and vancomycin show delayed equilibration between red cells and plasma (Lee et al., 1981a,b, 1984, 1986; Chen et al., 1983; Chang et al., 1988; Shin Wan et al., 1992). Schiff base formation with free fatty acid aldehyde groups in the membrane of RBCs was postulated to be the underlying mechanism.

C. Extent of Partitioning

Whereas information on the rate of partitioning is scarce, data on the extent of partitioning into RBCs have been reported for a larger number of drugs (Hinderling, 1988). With β-blockers and other compounds, lipophilicity was shown to be the single most important determinant for the extent of the RBC partitioning (Schanker et al., 1964; Holder and Hayes, 1965; Hinderling et al., 1984). Systematic investigations of the linearity of the extent of RBC partitioning were conducted only for a relatively small number of drugs (table 2). In some cases, the results showed medium concentration proportionate uptake (Hinderling et al., 1974; Veronese et al., 1980; Eckert and Hinderling, 1981; Roos and Hinderling, 1981; Hinderling, 1984; Shirkey et al., 1985; Ferrari and Cutler, 1990), in other cases, saturable uptake (Collste et al., 1976; Fleuren and Van Rossum, 1977; Wallace and Riegelman, 1977; Garrett et al., 1978; Bayne et al., 1981; Altmayer and Garrett, 1983; Niederberger et al., 1983; Brocks et al., 1984; San George et al., 1984; Urien et al., 1988; Beysens et al., 1991; Lin et al., 1992; Yatscoff et al., 1993a,b; Jusko et al., 1995; Snoek et al., 1996), and in still other cases, temperature- or pH-dependent drug distribution into RBCs (Holder and Hayes, 1965; Roos and Hinderling, 1981; Niederberger et al., 1983; Hinderling, 1984; Yatscoff and Jeffrey, 1987; Rudy and Poynor, 1990; Beysens et al., 1991; Yatscoff et al., 1993a; Reichel et al., 1994). Evidence for stereoselective RBC partitioning of racemic drugs has also been published (Lin et al., 1992; Chu et al., 1995a,b)

Only limited data are available on the impact of disease and demographic factors on the RBC partitioning of drugs. Significantly larger values for Ke/p,u were found in vitro for plasmodium-infected RBCs compared with

TABLE 2
Kinetics of red blood cell partitioning of drugs

Linear		Nonlinear	
Compound	Reference	Compound	Reference
Amiodarone	Veronese et al. (1980)	Acetazolamide	Collste et al. (1976)
Atropine	Eckert and Hinderling (1981)		Wallace and Riegelman (1977)
Chloroquine	Ferrari and Cutler (1990)	Chlorthalidone	Fleuren and Van Rossum (1977)
Digoxin and derivatives	Hinderling (1984a)	Cyclosporine	Niederberger et al. (1983)
Disopyramide	Hinderling et al. (1974)	Draflazine	Snoek et al. (1996)
Desethyl disopyramide	Hinderling et al. (1974)	Etofibrate	Altmayer and Garrett (1983)
Proquazone	Roos and Hinderling (1981)	Hydroxychloroquine	Brocks et al. (1984)
Valproate	Shirkey et al. (1985)	Indapamide	Urien et al. (1988)
		Mefloquine	San George et al. (1984)
		Methazolamide	Bayne et al. (1981)
		MK-927	Linn et al. (1992)
		Papaverine	Garrett et al. (1978)
		Rapamycin	Yatscoff et al. (1993a,b)
		Tacrolimus	Beysens et al. (1991)
			Jusko et al. (1995)

uninfected RBCs for mefloquine (Vidrequin et al., 1996) and chloroquine (Verdier et al., 1985). There was no difference in Ke/p,u for pethidine between young healthy subjects and old patients, suggesting that age had no influence on the RBC partitioning of the drug (Holmberg et al., 1982). For flucloxacillin and cloxacillin, Ke/p,u was similar in neonates, mothers, and controls (Herngren et al., 1982). Identical Ke/p,u values were also found for flucloxacillin in healthy subjects and in patients with pacemakers (Anderson et al., 1985). Similarly, Ke/p,u for phenobarbital was comparable in pregnant women at term and controls. However, Ke/p,u of phenobarbital was statistically significantly smaller in neonates than in the mothers (Walin and Herngren, 1985). Because phenobarbital is known to be bound to hemoglobin (Hilzenbecher, 1972), it may be speculated that the drug's affinity to fetal hemoglobin is decreased. In healthy subjects as well as in patients with renal or hepatic diseases, Ke/p was found to increase in proportion to the free fraction of valproic acid in plasma (Shirkey et al., 1985). This result suggested that Ke/p,u of the drug in healthy subjects and in the patients is similar. Likewise, similar Ke/p,u values were found for amobarbital, pentobarbital, and phenytoin in healthy volunteers and uremic patients (Ehrnebo and Odar-Cederlöf, 1975). Also, the Ke/p,u values for phenytoin in healthy subjects and cirrhotic or uremic patients were not different. No gender difference was found for the RBC partitioning of phenytoin. In patients with psoriasis, RBC uptake of dehydroepiandrosterone was reported to be smaller than in healthy subjects (Morsches et al., 1981).

Ex vivo and in vitro results indicate that coadministered acetazolamide is able to displace chlorthalidone bound to carbonic anhydrase in RBCs (Beerman et al., 1975). With other drug combinations, no competition for RBC binding sites was found (Ehrnebo and Odar-Cederlöf, 1977).

Reversibility of the RBC partitioning was shown for digoxin and derivatives, disopyramide, tetracycline, phenytoin, penicillin G, arbutin, p-nitrophenol and tacrolimus (Hinderling et al., 1974; Kornguth and Kunin, 1976; Ehrnebo and Odar-Cederlöf, 1977; Jun and Lee, 1980; Hinderling, 1984; Matsumoto et al., 1989; Beysens et al., 1991).

D. Binding Sites

The primary binding sites of drugs in the RBCs are associated with hemoglobin, proteins, or plasma membrane (table 3). The diuretics acetazolamide, methazolamide, and chlorthalidone and the ocular pressure reducing agent, dorzolamide, act as inhibitors of carbonic anhydrase and are bound extensively to this enzyme (Collste et al., 1976; Fleuren and Van Rossum, 1977; Wallace and Riegelman, 1977; Bayne et al., 1981; Lin et al., 1992; Biollaz et al., 1995), which is present in the RBC cytosol in seven isoforms and in larger concentrations than in the kidney (Maren, 1967). More than 90% of the amounts of this enzyme in the body are found in the RBCs. The immunosuppressive agents cyclosporin A and tacrolimus are strongly bound to the cytosolic proteins, cyclophilin and tacrolimus binding protein, in the RBCs (Agarwal et al., 1986; Hooks, 1994). The adenosine uptake inhibitor draflazine is bound extensively to the nucleoside transporters located on the RBCs (Snoek et al., 1996). Codeine, chlorpromazine, imipramine, mefloquine, and pyrimethamine have been shown to bind to the RBC plasma membrane (Bickel, 1975; San George et al., 1984; Rudy and Poynor, 1990; Mohammed et al., 1993). Digoxin and derivatives, sulfonamides, mefloquine, phenytoin, phenothiazines, barbiturates, phenylbutazone and derivative, salicylic acid and congeners, imipramine and derivatives, phenytoin, proquazone, pyrimethamine, as well as other drugs, are reportedly bound largely to hemoglobin (Hilzenbecher, 1972; Ber-

TABLE 3
Drug binding sites in red blood cells

Compound	Binding site	Reference
Chlorpromazine		Bickel (1975)
Codeine		Mohammed et al. (1993)
Imipramine	Plasma membrane	Bickel (1975)
Mefloquine		San George et al. (1984)
Pyrimethamine		Rudy and Poynor (1990)
Draflazine	Nucleoside transporter	Snoek et al. (1996)
Acetazolamide		Wallace and Riegelman (1977)
Chlorthalidone		Collste et al. (1976)
Dorzolamide	Carbonic anhydrase	Biollaz et al. (1995)
Methazolamide		Bayne et al. (1981)
MK-927		Lin et al. (1992)
Cyclosporin A	Cyclophilin	Agarwal et al. (1986)
Tacrolimus	Tacrolimus binding protein	Hooks (1994)
Aminophenzone		Hilzenbecher (1972)
Barbituates		Hilzenbecher (1972)
Chlordiazepoxide		Hilzenbecher (1972)
Digoxin and derivatives		Hinderling (1984a)
Imipramine and derivatives		Hilzenbecher (1972)
Mefloquine		San George et al. (1984)
Nitrofurantoin		Hilzenbecher (1972)
Oxyphenbutazone		Hilzenbecher (1972)
Phenothiazines	Hemoglobin	Hilzenbecher (1972)
Phenylbutazone		Hilzenbecher (1972)
Phenytoin		Hilzenbecher (1972)
Proquazone		Roos and Hinderling (1981)
Pyrimethamine		Rudy and Poynor (1990)
Salicylic acid and congeners		Hilzenbecher (1972)
Sulfinpyrazone		Hilzenbecher (1972)
Sulfonamides		Berneis and Boguth (1976)

neis and Boguth, 1976; Roos and Hinderling, 1981; Hinderling, 1984; San George et al., 1984; Rudy and Poynor, 1990).

Different types of interactions of drugs with proteins in RBCs have been observed. As mentioned above, many drugs bind to hemoglobin. Some of these drugs induce reversible allosteric changes in the hemoglobin molecule (Keidan et al., 1989; Venitz et al., 1996; Stone et al., 1992) or may, like acetylsalicylic acid and similar compounds, acetylate hemoglobin (Klotz and Tam, 1973; Zaugg et al., 1980; Massil et al., 1984). Inhibition of RBC carbonic anhydrase by acetazolamide and related compounds was mentioned above. Zifrosilone can reportedly inhibit cholinesterase in RBCs (Cutler et al., 1995).

V. Significance of Studying Red Blood Cell Partitioning of Drugs

A. Rational Choice of Biological Fluid (Whole Blood, Red Blood Cells, Plasma/Serum) for Assaying Drug Concentrations

The vast majority of drugs are assayed in plasma or serum. For drugs with Kb/p or Ke/p larger than 2.0, measuring concentrations in whole blood or erythrocytes rather than in plasma (or serum) increases the sensitivity of an assay with a given lower limit of quantitation (table 4; Fleuren and Van Rossum, 1977; Wallace and Riegelman, 1977; Bayne et al., 1981; Niederberger et al., 1983; Rambo et al., 1985; Winstanley et al., 1987; Hamberger et al., 1988; Nosál et al., 1988; Tett et al., 1988; Beysens et al., 1991; Jusko and D'Ambrosio, 1991; Yatscoff et al., 1993b; Chu et al., 1995b; Jusko et al., 1995; Kurokawa et al., 1996; Snoek et al., 1996). The increased sensitivity permits follow-up of the kinetics of drug for at least one additional half-life. Assaying concentrations in whole blood, rather than in plasma or serum, should also be considered with drugs showing significant temperature-dependent RBC partitioning (Wenk and Follath, 1983; Beysens et al., 1991; Yatscoff et al., 1993a). As a result of temperature-sensitive RBC partitioning, plasma concentrations of these drugs depend on the temperature maintained during centrifugation of the whole blood samples. Therefore, if these drugs are to be measured in plasma, the temperature during the centrifugation process must be kept at 37°C to reflect in vivo conditions. Alternatively, such drugs may be assayed in whole blood, obviating the need for temperature control of the samples. Indeed, most assays used today in the therapeutic monitoring of cyclosporin A and tacrolimus measure drug in whole blood (Wenk and Follath, 1983; Beysens et al., 1991).

With the neuroleptic drugs butaperazine, haloperidol, and thioridazine, the RBC concentrations reportedly correlate better with therapeutic effects or dose than plasma concentrations (Garver et al., 1977; Casper et al., 1980; Svensson et al., 1986). Based on these findings, measurement of RBC concentrations for therapeutic monitoring was recommended for butaperazine and haloperidol. The RBC concentrations of the metabolites of tricyclic antidepressants are reportedly the best toxicity markers for impaired intraventricular conduction (Ami-

TABLE 4
Drugs with large blood-to-plasma concentration ratios, Kb/p, in humans[a]

Class of drugs	Drug	Kb/p	Method	Reference
Inotrop/vasodilator	(+) Pimobendan	3.2[c]	ex vivo	Chu et al. (1995a)
	(−) Pimobendan	4.5[c]	ex vivo	Chu et al. (1995a)
Immunosuppressivum	Cyclosporin A	4[b]	in vitro, 20°C	Niederberger et al. (1983)
		2[b]	in vitro, 37°C	Niederberger et al. (1983)
		4.6[b]	ex vivo, 20°C	Hamberger et al. (1988)
		2.0[b]	ex vivo, 37°C	Kurokawa et al. (1996)
	Rapamycin	14.3[b]	in vitro, 37°C	Yatscoff et al. (1993b)
	Tacrolimus	22.6[b]	in vitro, 37°C	Beysens et al. (1991)
		29,[b] 55.5[b]	ex vivo, 37°C	Jusko et al. (1995)
				Jusko and D'Ambrosio (1991)
Carbonic anhydrase inhibitor	Acetazolamide	2.9[b,c]	in vitro, 37°C	Wallace and Riegelman (1977)
Diuretic	Dorzolamide	>100[b,c]	ex vivo	Biollaz et al. (1995)
	Methazolamide	241[b,c]	ex vivo	Bayne et al. (1981)
Thiazide-like diuretic	Chlorthalidone	30.7[b,c]	in vitro, 37°C	Fleuren and van Rossum (1977)
		32[b,c]	ex vivo	Fleuren and van Rossum (1977)
Antimalarial	Chloroquine	3.5, 3.2	in vitro, 20°C	Rambo et al. (1985)
				Nosàl et al. (1988)
	Desethylchloroquine	3.1	in vitro, 20°C	Rambo et al. (1985)
	Desethylamodiaquine	3.1	ex vivo	Winstanley et al. (1987)
Antirheumatic	Hydroxychloroquine	7.2	ex vivo	Tett et al. (1988)

[a] In accord with equation (4), the erythrocyte-to-plasma concentration ratios, Ke/p, exceed the corresponding Kb/p values of the listed compounds. Similarly, the red blood cell concentrations exceed the whole blood concentrations for the listed compounds, e.g., if Kb/p = 2.0, then Ke/p = 3.9, and the erythrocyte concentrations are 3.9 times larger than the whole blood concentrations.

[b] Saturable red blood cell partitioning; Kb/p measured at lower end of therapeutic plasma concentration range.

[c] Value estimated from data in reference literature.

tai et al., 1992). Similarly, the measurement of RBC concentrations has been recommended for lithium in evaluating adverse reactions and toxicity of the drug (Albrecht and Mueller-Oerlinghausen, 1976). With digoxin, RBC concentrations were found to better distinguish between toxic and nontoxic drug levels than did plasma concentrations (Kawai et al., 1982).

As mentioned above, the RBC partitioning of drugs may vary as a result of either pH-dependent binding to plasma proteins and/or RBCs. In this case, it may be more practical to measure drug concentrations in whole blood. This is true whether the blood samples are from subjects with normal blood pH or patients with alkalosis or acidosis. If the concentrations of such drugs are measured in plasma, care must be taken that the pH of the blood samples is controlled during centrifugal separation of RBCs and plasma. However, to understand the pharmacokinetics of pH-sensitive drugs in patients with abnormal blood pH, it is necessary to measure concentrations in both plasma and RBCs.

When considering assaying concentrations of drugs in whole blood, possible degradation by enzymes located in the RBCs must be excluded. As mentioned above, RBCs have been shown to contain various enzymes that can effectively metabolize drugs. As a general rule, stability of drugs should be determined routinely in whole blood, plasma, or serum, independent of which matrix is used for assaying drug concentrations.

Choosing whole blood as the biological fluid for measuring the concentrations of drug does not obviate the need for studying rate and extent of partitioning of drug from plasma into RBCs. Knowing the type of kinetics of drug RBC partitioning (linear or nonlinear) and the time to equilibration is mandatory for a correct interpretation of kinetic parameters derived from concentrations measured in whole blood. The above examples indicate that knowledge of drug partitioning into the cellular constituents of blood and familiarity with the covariates impacting this process are critical in choosing the appropriate assay matrix for drug.

B. Significance of Rate and Extent of Red Blood Cell Partitioning for Physiological Interpretation of Organ Clearances and Volumes of Distribution

As mentioned in Section V.A., most often, drug concentrations are measured in plasma or serum and not in whole blood; hence, plasma or serum is the reference fluid for the derived pharmacokinetic parameters such as clearances and volumes of distribution. However, whole blood, not plasma or serum, is flowing through the vessels of the human body. Therefore, it would appear that whole blood rather than plasma is the more appropriate reference fluid for calculating and interpreting clearances and volumes of distribution. In agreement with this rationale, routine determination of the whole blood to plasma concentration ratio was recently proposed for drugs under development (Peck et al., 1992).

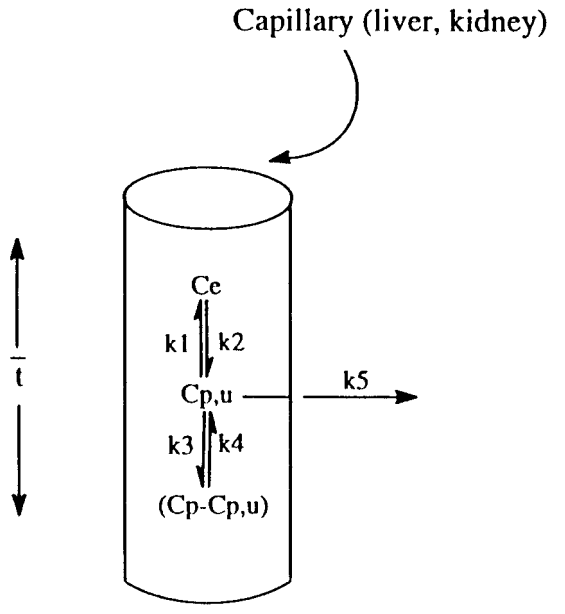

FIG. 2. Multiple equilibria between bound/partitioned and unbound concentrations of drug in whole blood and significance of forward and backward rate constants for physiological interpretation of organ clearances. Ce, drug concentration in red blood cells; Cp,u, unbound drug concentration in plasma water; Cp, drug concentration in plasma; (Cp-Cp,u), bound drug concentration in plasma; k1, rate constant of partitioning of free drug from plasma water into red blood cells; k2, rate constant of repartitioning of drug from the red blood cells into plasma water; k3, rate constant of binding of free drug in plasma water to plasma proteins; k4, rate constant of debinding of drug bound to plasma proteins; k5, rate constant of elimination of drug from plasma water; $\bar{t}$, mean transit time of blood in capillaries of liver ($\bar{t}$ = 2.5 seconds) or kidney ($\bar{t}$ = 28 seconds).

It is commonly assumed that only unbound drug molecules in the plasma water phase of blood can leave the capillary bed in the liver and kidney for elimination (fig. 2; Wilkinson, 1987). Most drugs are bound to some extent to plasma proteins and/or partitioned into RBCs. For such drugs, bound, partitioned, and unbound fractions in blood coexist and equilibria are maintained between the free and bound species. In contrast to the unbound molecules, the bound or partitioned molecules are not immediately available for elimination because they cannot leave the capillary bed in eliminatory organs.

A mean transit time of capillary blood in the liver of 10 seconds was estimated in animal experiments (Goresky, 1963). Corresponding values of 2.5 seconds and 28 seconds were found for cortex and medulla of the kidney in dogs (Kramer et al., 1960). Free drug molecules can leave the capillaries in liver and kidney during these times. As a consequence of the equilibria between the free and bound drug species in whole blood, unbound drug molecules that undergo elimination may be replaced by previously bound or partitioned drug molecules, which as unbound molecules can now leave the capillaries of the liver and kidney. The efficiency of resupply of free drug by previously bound or partitioned

molecules depends on the magnitude of the binding/partitioning and debinding/repartitioning processes, relative to the above residence times of blood in the capillaries of liver and kidney (fig. 2). If repartitioning of drug with significant distribution into RBCs (Ke/p > 0.25) is fast compared with the residence time of blood in the capillaries, significant amounts of previously partitioned drug molecules may be eliminated during each passage of blood through the liver or kidney. However, if the repartitioning of drug is slow compared with the capillary blood's residence time, then negligible amounts of drug previously bound to blood constituents will be eliminated during each passage of blood through the liver or kidney. Only in the former case is whole blood the appropriate reference fluid with physiological relevance. In choosing the appropriate reference fluid for computing renal clearance, consideration must also be given to the possibility of pretubular deviation of a portion of RBCs by a "skimming mechanism" (Pappenheimer and Kinter, 1956; Kinter and Pappenheimer, 1956a,b). The above discussion assumes that debinding of drug from plasma proteins is fast compared with the residence time of blood in the capillaries of eliminatory organs.

Evidence obtained from in vivo studies and in vitro experiments in humans, and additional investigations using isolated rat liver and kidney perfusion techniques, indicates that the time for equilibration between plasma and RBCs relative to the mean transit time of blood in the capillaries of liver or kidney is in the same range for some compounds (Reichel et al., 1994; Morgan et al., 1996), but is much slower for a distinct number of other drugs (table 5; Schanker et al., 1961, 1964; Goresky et al., 1975; Kornguth and Kunin, 1976; Wallace and Riegelman, 1977; Skalski et al., 1978; Noel, 1979; Jun and Lee, 1980; Lee et al., 1981a,b, 1984, 1986; Tucker et al., 1981; Chen et al., 1983, 1992; Hinderling, 1984; Wiersma et al., 1984; Chang et al., 1988; Lee and Chiou, 1989a,b; Matsumoto et al., 1989; Beysens et al., 1991; Shin Wan et al., 1992; Rolan et al., 1993; Hasegawa et al., 1996). For these latter drugs, calculating clearance referenced to drug concentration in whole blood from

$$Clb = Clp/Kb/p, \quad [14]$$

where Clp is the organ clearance (renal or hepatic) referenced to drug concentration in plasma, is unwarranted. This becomes more evident from equation (15), which is obtained by inserting equation (4) into equation (14):

$$Clb = Clp/[(1 - Hc) + Ke/p \cdot Hc] \quad [15]$$

Equation (15) indicates the variables impacting organ clearances, which are referenced to drug concentrations in whole blood. Equation (15) is only valid if Ke/p is constant across the eliminatory organ (kidney or liver) (Stec et al., 1979; Lee et al., 1980). This means that reequilibration between the concentrations in the RBCs and plasma for drugs with significant RBC partitioning occurs quickly, relative to the transit time of blood in the capillaries. Equations (14) and (15) indicate that except if Ke/p = Kb/p = 1.0, the values for Clp and Clb differ. For drugs with Ke/p or Kb/p > 1.0, Clp exceeds Clb, and for compounds with Ke/p or Kb/p < 1.0, Clb exceeds Clp.

Experimental limitations in humans may preclude a definitive assignment of the appropriate biological fluid of reference in calculating hepatic or renal clearance for a drug. However, the correct biological fluid of reference may be identified for dialysis clearance. In this case, all the critical variables (Ce, Cp, and Hc) can be determined in the arterial and venous lines of the dialyzer. Provided Ke/p and Hc are found to be constant, whole blood is the appropriate biological fluid of reference. It is mandatory to measure Ce and Cp in the blood samples obtained from the predialyzer and postdialyzer lines within a few seconds after drawing to conclusively demonstrate constancy of Ke/p across the dialyzer.

The concept of referencing pharmacokinetic parameters of appropriate drugs to whole blood, as the physiologically meaningful body fluid, has been applied also to intercompartmental clearances and volumes of distribution (Stec and Atkinson, 1981; Odeh et al., 1993). Experimental evidence in support of fast equilibration of drug between RBCs and plasma must be available, like for the above parameters, to justifiably reference intercom-

TABLE 5
Drugs with slow rates of red blood cell partitioning in humans[a]

Drug	Reference
Acetazolamide	Wallace and Riegelmann (1977)
Arbutine	Matsumoto et al. (1989)
Bumetamide	Chang et al. (1988)
Creatinine	Skalski et al. (1978)
Darstine	Schanker et al. (1961)
Desethyldorzolamide	Hasegawa et al. (1996)
Digoxigenin digitoxoside	Hinderling (1984a)
Digoxin-16′-glucuronide	Hinderling (1984a)
Epinephrine	Schanker et al. (1961)
Gentamycin	Lee et al. (1981a)
Hippuric acid	Schanker et al. (1964)
Metformin	Noel (1979)
	Tucker et al. (1981)
Norepinephrine	Schanker et al. (1961)
p-Aminohippuric acid	Schanker et al. (1961)
Papaverine	Garrett et al. (1978)
Penicillin G	Kornguth and Kunin (1976)
	Matsumoto et al. (1989)
Phenol red	Schanker et al. (1964)
Serotonin	Schanker et al. (1961)
Sulfosalicylic acid	Schanker et al. (1964)
Tacrolimus	Beysens et al. (1991)
Tetracycline	Jun and Lee (1980)
Tucaresol	Rolan et al. (1993)
Vancomycin	Shin et al. (1992)

[a] Time to 90% of equilibrium between red blood cells and plasma or buffer >30 seconds. Only drugs with significant red blood cell partitioning are considered.

partmental clearances and volumes of distribution to whole blood.

C. Red Blood Cells as Probes for in Vivo Drug Distribution

A highly significant log-log linear correlation between Ke/p,u and the steady-state volume of distribution referenced to the unbound drug concentration in plasma water, Vu,ss, for 38 cationic drugs in humans has been reported (fig. 3; Hinderling, 1988). The data base for these drugs consisted of pairs of Ke/p,u values determined in vitro or ex vivo and Vss values obtained in vivo. It was assumed that the values obtained for Ke/p,u in vitro and ex vivo were equivalent. Values for Vu,ss were computed from Vu,ss = Vss/fu, where fu corresponds to the fraction of drug unbound in plasma water determined in vitro or ex vivo. Using this database, predictions of Vu,ss from Ke/p,u values were estimated to have a mean error of 50% and a bias of 2%. This magnitude of prediction error is small enough to make these estimates of Vu,ss useful in practice. Reportedly, highly significant log-log correlations also exist between Vu,ss and the n-octanol/water partition coefficient, P, for the 38 cationic drugs tested, suggesting that lipophilicity of the compounds is most important for the extent of drug distribution in the body and that electronic properties were not relevant.

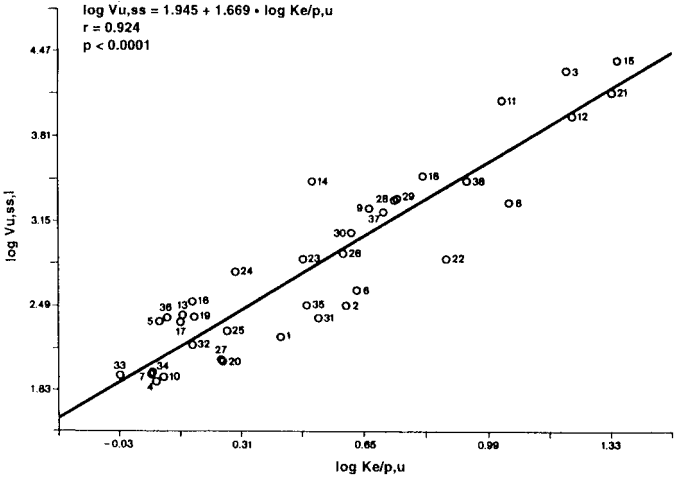

FIG. 3. Correlation between in vivo steady-state volume of distribution referenced to the unbound drug fraction in plasma and in vitro red blood cells to plasma water partition coefficient for 38 cationic drugs. Ke/p,u, red blood cell to plasma water partition coefficient for drug determined in vitro; Vu,ss, steady-state volume of distribution referenced to the unbound drug concentration in plasma water determined in vivo. 1, acebutolol; 2, alprenolol; 3, amitryptiline; 4, atenolol; 5, atropine; 6, chlordiazepoxide; 7, cimetidine; 8, diazepam; 9, diltiazem; 10, disopyramide; 11, haloperidol; 12, imipramine; 13, lidocaine; 14, lorcainide; 15, maprotiline; 16, mepivacaine; 17, metoprolol; 18, midazolam; 19, morphine; 20, N-acetylprocainamide; 21, nortryptiline; 22, papaverine; 23, pentazocine; 24, pethidine; 25, pindolol; 26, prazosin; 27, procainamide; 28, propranolol; 29, proquazone; 30, quinidine; 31, quinine; 32, flumazenil; 33, SM 1213; 34, sotalol; 35, tiapamil; 36, timolol; 37, tolamolol; 38, verapamil. Reproduced with permission from Hinderling (1988).

With drugs having volumes of distribution significantly larger than the volume of blood or plasma, the largest fraction of an administered dose is likely to be distributed into skeletal muscle, which constitutes approximately 40% of the total body mass (Fichtl and Kurz, 1978; Clausen and Bickel, 1993). Among muscular tissue proteins, actin and myosin constitute the largest fraction and were found to bind drugs significantly (Kurz and Fichtl, 1983). RBC partitioning and hemoglobin binding as well as muscular tissue binding were found to primarily depend on the lipophilicity of a drug (Schanker et al., 1961, 1964; Kurz and Fichtl, 1983; Hinderling et al., 1984; Hinderling, 1988), and statistically significant correlations were reported to exist between the binding of drugs to hemoglobin and muscular tissue (Kurz and Fichtl, 1983). It can be speculated that successful predictions of in vivo drug distribution from in vitro RBC partitioning are due to the relevance of lipophilicity for both processes.

D. Use of Red Blood Cell Suspensions in Plasma and Buffer to Determine Plasma Protein Binding of Drugs

The RBC partitioning technique can also be used as an alternative method to determine plasma protein binding (Garrett and Hunt, 1974; Veronese et al., 1980; Cenni et al., 1995). The experiments should be conducted under physiological conditions, i.e., at 37°C and pH 7.4. With this method, separate RBC suspensions in plasma and plasma water (or buffer) are prepared from blood samples obtained from individual subjects. After spiking with drug, equilibration, and subsequent centrifugal separation of the RBCs, the respective drug concentrations in RBCs, plasma, and plasma water are determined (fig. 1). The values for Ke/p and Ke/p,u are then computed, and the fraction of drug unbound in plasma is obtained from fu = (Ke/p)/(Ke/p,u) after rearrangement of equation (3). Minimal drug binding to and/or partitioning into RBCs, as well as absence of drug metabolism by the RBCs, are preconditions for the successful application of this alternative methodology to determine plasma protein binding.

RBC suspensions in plasma or buffer spiked with drug can be considered as a biological equilibrium dialysis (BED) system (Hinderling, 1987). The advantage of the BED system over the classical equilibrium dialysis system, using two chambers separated by an artificial semipermeable membrane (AED), is that the RBC membranes have a much larger surface. As a result, equilibration of drug in the BED system is much more quickly established than with the AED system (Hinderling, 1987). A comparison of the results on the equilibration time required for 22 acidic, basic, or nonelectrolytic drugs showed that the estimated equilibration time ranged between 2 and 45 minutes for the BED system and between 120 and 960 minutes for the AED system. As a result, water shift, change in pH, and degradation of plasma proteins, phenomena known to occur during

prolonged equilibrium dialyses, are less likely to occur with the BED system than with the AED system (Hinderling, 1987). Excellent agreement was found between the respective estimates for the unbound fraction of drug in plasma by the two methods for 22 drugs with fu values ranging from 0.01 to 1.0 (fig. 4). Similar results were obtained in comparing the fu estimates by the BED method and ultrafiltration for five drugs (Ho Ncoc-Ta Truong et al., 1984).

A modified BED method employing RBC suspensions in variably diluted plasma has been shown to allow measurements of protein binding of very lipophilic drugs like tetrahydrocannabinol, amiodarone, and etretinate, which bind significantly to artificial membranes rendering conventional equilibrium dialysis (AED) and ultrafiltration methods impractical (Garrett and Hunt, 1974; Veronese et al., 1980; Urien et al., 1992a,b; Cenni et al., 1995).

E. Significance of Studying the Effects of Drugs on Red Blood Cells

Screening drugs for beneficial and/or toxic effects on RBCs should be done early in drug development. Some of these investigations may be conducted in vitro with human RBCs. Beneficial effects of drugs include schizontocidal activity in parasitized RBCs, enhanced dissociation of O_2 from hemoglobin, increased RBC deformability, decreased sickling of RBCs containing hemoglobin S, and inhibition of adenosine uptake into RBCs (Orringer et al., 1986; Keidan et al., 1989; Ambrus et al., 1990; Stone et al., 1992; Charache et al., 1995; Cenni and Betschart, 1995; Snoek et al., 1996; Venitz et al., 1996). Toxic effects of drugs include increased formation of methemoglobin and hemolysis (Grossman and Jollow, 1988; Pirmohamed et al., 1991; Fasanmade and Jusko, 1995). RBCs have been employed as surrogates for endothelial cells to study the pharmacodynamics of draflazine, an adenosine uptake inhibitor with potentially cardioprotective properties (Snoek et al., 1996). Historically, RBCs were successfully employed as models for cardiac myocytes in elucidating the molecular mechanism of action of cardiac glycosides (Schatzmann, 1953), and RBCs were more recently used as surrogates for body cells in general in studying the effects of tolrestat and other antidiabetics (Kuusisto et al., 1994; Van Griensven et al., 1995). Modeling was successfully applied in correlating the pharmacodynamics measured in the RBCs with the pharmacokinetics of some of these drugs (Fasanmade and Jusko, 1995; Van Griensven et al., 1995). Toxicity of primaquine and other drugs inducing massive denaturation of hemoglobin can be predicted to occur in subjects with glucose-6-phosphate dehydrogenase deficiency (Luzzatto, 1995). Decrease in ocular pressure and diuretically active carbonic anhydrase inhibitors can be identified in vitro by using RBC partitioning methods.

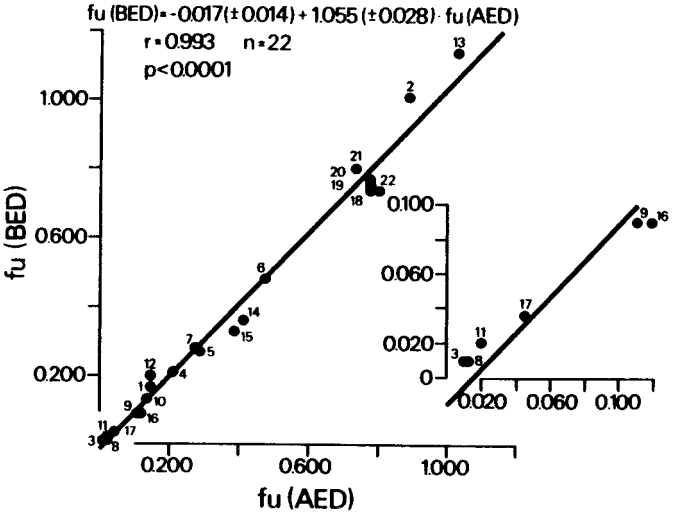

FIG. 4. Plot of the unbound fraction of drug in plasma by the red blood cell partitioning method, fu (BED), against the corresponding value obtained by the traditional equilibrium dialysis method, fu (AED). fu: fraction of drug unbound in plasma water, AED, Equilibrium dialysis method employing artificial membranes, BED, Equilibrium dialysis method using biological membranes. 1, amitriptyline; 2, atropine; 3, binedaline; 4, fentanyl; 5, imipramine; 6, lidocaine; 7, minaprine; 8, nicardipine; 9, nortriptyline; 10, propranolol; 11, proquazone; 12, quinidine; 13, tropine; 14, amobarbital; 15, pentobarbital; 16, phenytoin; 17, digitoxin; 18, dihydrodigoxin; 19, digoxin; 20, digoxigeninbisdigitoxoside; 21, digoxigeninmonodigitoxoside; 22, β-methyldigoxin. Reproduced with permission from Hinderling (1987).

It is self-evident that routine determination of RBC partitioning of drugs in early stages of development can effectively aid in screening for candidates with the above or other beneficial or toxic effects on RBCs. Hence, it is surprising to note that the study of the partitioning of drugs into RBCs and the other cellular constituents of blood is not a standard part of routine investigations performed in screening drugs for potential beneficial or toxic effects.

F. Red Blood Cells as Markers

Myelosuppressive toxicity of azathioprine and 6-mercaptopurine is reportedly negatively correlated with thiopurine methyltransferase activity in the RBCs in leukemic and immunosuppressed patients (Lennard et al., 1990; Lennard, 1992). The determination of the activity of thiomethyltransferases and other polymorphic methyltransferases and monomorphic acetylases in RBCs has been proposed as a means of characterizing the metabolizer status of patients after significant correlations of the respective activities of these enzymes in the RBCs and in the liver or kidneys were demonstrated (Woodson et al., 1982; Scott et al., 1988; Szumlanski et al., 1992; Hutabarat et al., 1994). Different types of essential hypertension can reportedly be differentiated based on the type of ion transport defects found in the RBC membranes of hypertensive subjects (Garay et al., 1994; Duhm and Engelmann, 1992).

VI. Conclusions

It is surprising that in studying the disposition kinetics of drugs in blood and its subcompartments over the last 30 years, the study of RBC partitioning of drugs has received much less attention than the plasma protein binding of drugs. As a consequence, there are many opportunities for challenging research in this underdeveloped area.

It is currently routine practice to develop assays for drug concentration measurements in plasma or serum. In only a few instances have assays been developed to measure drugs in whole blood. However, the choice of the appropriate assay matrix is not always rationally based, and factors such as magnitude, pH, and temperature dependency of RBC partitioning are rarely considered in the decision-making process.

The goal of any drug concentration measurement in a blood, plasma, or serum sample must be to have the measured value precisely reflect the drug concentration that existed under in vivo conditions at the time of sampling. It is clear that the RBC partitioning of drugs varies: with some drugs, it is a fast process, with others, equilibration between plasma and RBCs is significantly delayed. With most drugs, RBC partitioning is temperature-dependent. A pH dependency of RBC partitioning has been observed with a number of compounds. Furthermore, some drugs' interaction with RBCs may result in Schiff base formation. RBCs contain various enzymes that can metabolize many drugs. Thus, the handling of blood samples after collection should be done in accord with a protocol that is based on the results of previous studies, which establish the interaction between RBCs and the drug under development. Sample protocols should specify time to centrifugation, temperature, and pH conditions, as well as need for addition of suitable enzyme inhibitors.

The evidence presented in this review warrants a change from current practice in assay development and validation. Currently, the systematic investigation of drug distribution into and the possible metabolism by the RBCs is neglected. Protocols for the handling and storage of blood samples after their withdrawal should be established. The choice of the appropriate assay matrix should be rationally based.

Additional benefits that would result from a systematic study of the interaction between drugs and RBCs include: (*a*) a rationally derived and physiologically correct reference concentration of the drug for computing organ clearances, i.e., whole blood, plasma or serum; (*b*) estimates for the in vivo distribution of new cationic drugs; (*c*) use of the RBC partitioning procedure as an alternative method to determine plasma protein binding of drugs, particularly highly lipophilic drugs; and (*d*) early determination of desirable and undesirable effects of drugs on RBCs.

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REFERENCES

AGARWAL, R. P., MCPHERSON, R. A., AND THREATTE, G. A.: Evidence for a cyclosporin-binding protein in human erythrocytes. Transplantation **42:** 627–632, 1986.

ALBRECHT, J., MUELLER-OERLINGHAUSEN, B.: Zur klinischen Bedeutung der intraerythrocytaeren Lithiumkonzentration: Ergebnisse einer katamnestischen Studie. Drug Res. **26:** 1145–1147, 1976.

ALTMAYER, P., AND GARRETT, E. R.: Plasmolysis, red cell partitioning, and plasma binding of etofibrate and their degradation products. J. Pharm. Sci. **72:** 1309–1318, 1983.

AMBRUS, J. L., ANAIN, J. M., ANAIN, S. M., ANAIN, P. M., ANAIN, J. M., JR., STADLER, S., MITCHELL, P., BROBST, J. A., COBERT, B. L., AND SAVITZKY, J. P.: Dose-response effects of pentoxyphylline on erythrocyte filterability: clinical and animal model studies. Clin. Pharmacol. Ther. **48:** 50–56, 1990.

AMITAI, Y., ERICKSON, T., KENNEDY, E. J., LEITEIN, J. B., HRYHORCZUK, D. O., NOBLE, J., HANASHIRO, P. K., AND FISHER, H.: Tricyclic antidepressants in red cells and plasma: correlation with impaired intraventricular conduction. Clin. Pharmacol. Ther. **54:** 219–227, 1992.

ANDERSON, P., BLUHM, G., EHRNEBO, M., HERNGREN, L., AND JACOBSON, B.: Pharmacokinetics and distribution of flucloxacillin in pacemaker patients. Eur. J. Clin. Pharmacol. **27:** 713–719, 1985.

ARMSTRONG, J. A., SLAUGHTER, S. E., MARKS, G. S., AND ARMSTRONG P. W.: Rapid disappearance of nitroglycerin following incubation with human blood. Can. J. Physiol. Pharmacol. **58:** 458–462, 1980.

AXELROD, J., AND COHN, C. K.: Methyltransferase enzymes in red blood cells. J. Pharmacol. Exp. Ther. **176:** 650–654, 1971.

BACK, D. J., ORMESHER, S., TJIA, J. F., AND, MACLEOD, R.: Metabolism of 2′,3′-dideoxyinosine (ddI) in human blood. Br. J. Clin. Pharmacol. **23:** 319–322, 1992.

BARRY, M., BACK, D., ORMESHER, S., BEECHING, N., AND NYE, F.: Metabolism of didanosin (ddI) by erythrocytes: pharmacokinetic implications. Br. J. Clin. Pharmacol. **36:** 87–88, 1993.

BAYNE, W. F., TAO, T. F., ROGERS, G., CHU, L. C., AND THEEUWES, F.: Time course and disposition of methazolamide in human plasma and red blood cells. J. Pharm. Sci. **70:** 75–80, 1981.

BEERMAN, B., HELLSTROEM, K., LINDSTROEM, B., AND ROSEN, A.: Binding-site interaction of chlorthalidone and acetazolamide, two drugs transported by red cells. Clin. Pharmacol. Ther. **17:** 424–432, 1975.

BELZ, G. G., VOLLMER, K. O., AND WISSLER, J. H.: Zur Hemmwirkurg von Herzglykosiden auf die ^{86}Rb-Aufnahme der Erythrocyten. Eur. J. Clin. Pharmacol. **4:** 92–98, 1972.

BENJAMIN, M. A., AND DUNHAM, P. B.: Asymmetry of Na/K cotransport in human erythrocytes. J. Gen. Physiol. **82:** 27a–28a, 1983 (abstract).

BENNETT, B. M., BRIEN, J. F., NAKATSU, K., AND MARKS, G. S.: Role of hemoglobin in the differential biotransformation of glyceryltrinitrate and isosorbide dinitrate by human erythrocytes. J. Pharmacol. Exp. Ther. **234:** 228–232, 1985.

BENNETT, B. M., TWIDDY, D. A. S., MOFFAT, J. A., ARMSTRONG, P. W., AND MARKS, G. S.: Sex-related difference in the metabolism of isosorbide dinitrate following incubation in human blood. Biochem. Pharmacol. **24:** 3729–3734, 1983.

BERGQVIST, Y., AND DOMEIJ-NYBERG, B.: Distribution of chloroquine and its metabolite desethylchloroquine in human blood cells and its implication for the quantitative determination of these compounds in serum and plasma. J. Chromatogr. Biomed. Appl. **272:** 137–148, 1983.

BERNEIS, K., AND BOGUTH, W.: Distribution of sulfonamides and sulfonamide potentiators between red cells, proteins and aqueous phases of the blood of different species. Chemotherapy **22:** 390–409, 1976.

BEUTLER, E., LICHTMAN, M. A., COLLER, B. S., AND KIPOS, T. J., EDS: Williams Hematology, pp. 349–363, McGraw Hill, Inc., New York, 1995a.

BEUTLER, E., LICHTMAN, M. A., COLLER, B. S., AND KIPOS, T. J., EDS: Williams Hematology, pp. 394–417, McGraw Hill, Inc., New York, 1995b.

BEYSENS, A. J., WIJNEN, R. M. H., BEUMAN, G. H., VANDER HERDEN, J., KOOTSTRA, G., AND VAN AS, H.: FK 506: monitoring in plasma or whole blood? Transplant. Proc. **23:** 2745–2747, 1991.

BICKEL, M. H.: Binding of chlorpromazine and imipramine to red cells, albumin, lipoproteins and other blood components. J. Pharm. Pharmacol. **27:** 733–738, 1975.

BIOLLAZ, J., MUNAFO, A., BUCLIN, T., GERVASONI, J-P., MAGNIN, J-L., JAQUET, F., AND BRUNNER-FERBER, F.: Whole blood pharmacokinetics and metabolic effects of the topical carbonic anhydrase inhibitor dorzolamide. Eur. J. Clin. Pharmacol. **47:** 453–460, 1995.

BLONDHEIM, S. H.: Acetylation by blood cells. Arch. Biochem. Biophys. **55:** 365–372, 1955.

BONATE, P. L., AND PEYTON, A.: Uptake and biodisposition of 2-ylcyanamide-

1,3,4-thiadiazole (LY 217896) by red blood cells. Biopharm. Drug Dispos. **16:** 151–167, 1995.
BORGÅ, O., AND LINDBERG, C.: Pharmacokinetic implications of slow equilibration of terbutaline between plasma and erythrocytes. Eur. J. Respir. Dis. **65(Suppl 134):** 73–80, 1984.
BOWYER, F.: The kinetics of the penetration of nonelectrolytes into the mammalian erythrocyte. Int. Rev. Cytol. **6:** 469–511, 1957.
BROCKS, D. R., SKEITH, K. J., JOHNSTON, C., EMAMIBAFRAMI, J., DAVIS, P., RUSSELL, A. S., AND JAMALI, F.: Hematologic disposition of hydroxychloroquin enantiomers. J. Clin. Pharmacol. **34:** 1088–1097, 1984.
BRYCE, T. A., AND BURROWS, J. L.: Determination of oxypentifylline and a metabolite, 1-(5′-hydroyhexyl)-3,7-dimethylxanthine, by gas-liquid chromatography using a nitrogen-selective detector. J. Chromatogr. **181:** 355–361, 1980.
CALVO, R., CARLOS, R., AND ERILL, S.: Effects of disease and acetazolamide on procaine hydrolysis by red blood cell enzymes. Clin. Pharmacol. Ther. **27:** 179–183, 1980.
CARRUTHERS, A., AND MELCHIOR, D. L.: Effects of lipid environment on membrane transport: the human erythrocyte sugar transport protein lipid/bilayer system. Annu. Rev. Physiol. **50:** 257–271, 1988.
CASPER, R., GARVER, D. L., DEKIRMENJIAN, H., CHANG, S., AND DAVIS, J.: Phenothiazine levels in plasma and red blood cells. Arch. Gen. Psychiatry **37:** 301–305, 1980.
CENNI, B., AND BETSCHART, B.: The in vitro distribution of halofantrine in human blood and Plasmodium falciparum parasitized red bood cells. Chemotherapy **41:** 153–158, 1995.
CENNI, B., MEYER, J., BRANDT, R., AND BETSCHART, B.: The antimalarial drug halofantrin is bound mainly to low and high density lipoproteins in human serum. Br. J. Clin. Pharmacol. **39:** 519–526, 1995.
CHALMERS, A. H., KNIGHT, P. R., AND ATKINSON, M. R.: Conversion of azathioprine into mercaptopurine and mercaptoimidazole derivatives in vitro and during immunosuppressive therapy. Aust. J. Exp. Biol. Med. Sci. **45:** 681–691, 1967.
CHAN, M. Y., WONG, B. Y. M., YAU, K. W., AND TAM, W. Y. K.: Haloperidol reductase activity in the red blood cells of chronic schizophrenic patients and normal subjects. Asia Pac. J. Pharmacol. **7:** 57–60, 1992.
CHANG, M. W., YOON, E. J., LEE, M. G., AND KIM D. K.: Pharmacokinetics of drugs in blood VI: unusual distribution and storage effect of bumetamide. Seoul Univ. J. Pharm. Sci. **13:** 1–7, 1988.
CHARACHE, S., TERRIN, M. L., MOORE, R. D., DOVER, G. J., BARTON, F. B., ECKERT, S. V., MCMAHON, R. P., BONDS, D. R., AND THE INVESTIGATORS OF THE MULTICENTER STUDY OF HYDROXYUREA IN SICKLE CELL ANEMIA: Effect of hydroxyurea on the frequency of painful attacks in sickle cell anemia. N. Engl. J. Med. **332:** 1317–1322, 1995.
CHEN, M. L., LEE, M. G., AND CHIOU, W. L.: Pharmacokinetics of drugs in blood: III—metabolism of procainamide and storage effect of blood samples. J. Pharm. Sci. **72:** 572–574, 1983.
CHEN, T. M., ABDELHAMEED, M. H., AND CHIOU, W. L.: Erythrocytes as a total barrier for renal excretion of hydrochlorothiazide: slow influx and efflux across erythrocytes membranes. J. Pharm. Sci. **81:** 212–218, 1992.
CHONG, S., AND FUNG, H-L.: Kinetic mechanisms for the concentration dependency of in vitro degradation of nitroglycerin and glyceryl dinitrates in human blood: metabolite inhibition or cosubstrate depletion? J. Pharm. Sci. **78:** 295–302, 1989.
CHU, K-M., SHIEH S-M., AND HU, O. Y-P.: Pharmacokinetics and pharmacodynamics of enantiomers of pimobendan in patients with dilated cardiomyopathy and congestive heart failure after single and repeated dosing. Clin. Pharmacol. Ther. **57:** 610–621, 1995a.
CHU, K-M., SHIEH, S-M., AND HU, O. Y-P.: Plasma and red blood cell pharmacokinetics of pimobendan enantiomers in healthy Chinese. Eur. J. Clin. Pharmacol. **47:** 537–542, 1995b.
CLAUSEN, J., AND BICKEL, M. H.: Prediction of drug distribution in distribution dialysis and in vivo from binding to tissues and blood. J. Pharm. Sci. **82:** 345–349, 1993.
COLLSTE, P., GARLE, M., RAWLINS, M. D., AND SJOEQVIST, F.: Interindividual differences in chlorthalidone concentration in plasma and red cells of man after single and multiple dose. Eur. J. Clin. Pharmacol. **9:** 319–325, 1976.
COSSUM, P. A.: Role of the red blood cell in drug metabolism. Biopharm. Drug Dispos. **9:** 321–336, 1988.
COSSUM, P. A., AND ROBERTS, M. A.: Nitroglycerin disposition in human blood. Eur. J. Clin. Pharmacol. **29:** 169–175, 1985.
COSTELLO, P. B., CARVANA, J. A., AND GREEN, P. A.: The relative roles of hydrolases of the erythrocyte and other tissues in controlling aspirin survival in vivo. Arthritis Rheum. **27:** 422–426, 1984.
CUTLER, N. R., SEIFERT, R. D., SCHLEMAN, M. R., SRAMEK, J. J., SZYLLEIKO, O. J., HOWARD, D. R., BARCHOWSKI, A., WARDLE, T., AND BRASS, E. P.: Acetylcholinesterase inhibition by zifrosilone: pharmacokinetics and pharmacodynamics. Clin. Parmacol. Ther. **58:** 54–61, 1995.
DANNON, A., AND SAPIRA, J. D.: Uptake and metabolism of catecholamines by the human red cell. Clin. Pharmacol. Ther. **13:** 916–922, 1972.
DELAUNAY, J.: Genetic disorders of the red cell membrane. Crit. Rev. Oncol. Hematol. **19:** 79–110, 1995.
DEUTICKE, B.: Properties and structural basis for simple diffusion pathways in the erythrocyte membrane. Rev. Physiol. Biochem. Pharmacol. **78:** 1–97, 1977.
DICARLO, F. J., HARTIGAN, J. M., JR., AND PHILLIPS, G. E.: Enzyme degradation of pentaerythritol tetranitrate by human blood. Proc. Soc. Exp. Biol. Med. **118:** 514–516, 1965.
DIEM, K., AND LENTNER, C.: Documenta Geigy, Scientific Tables, Geigy Pharmaceuticals, (Ciba-Geigy Ltd.), ed. 7, pp. 555–561, 1975a.
DIEM K., AND LENTNER C.: Documenta Geigy, Scientific Tables, Geigy Pharmaceuticals, (Ciba-Geigy Ltd.), ed. 7, p. 612, 1975b.
DIEM, K., AND LENTNER, C.: Documenta Geigy Scientific Tables, Geigy Pharmaceuticals (Ciba-Geigy Ltd.), ed. 7, pp. 617–618, 1975c.
DRAYER, D. D., STRONG, J. M., JONES, B., SANDLER, A., AND REIDENBERG, M.: In vitro acetylation of drugs by human blood cells. Drug Metab. Dispos. **2:** 499–505, 1974.
DUHM, J., AND ENGELMANN, B.: Na^+-Li^+ countertransport of erythrocytes and cardiovascular pathophysiology: the lipid hypothesis. Pharm. Pharmacol. Lett. **2:** 32–33, 1992.
ECKERT, K. G.: The metabolism of aminophenols in erythrocytes. Xenobiotica **18:** 1319–1326, 1988.
ECKERT, M., AND HINDERLING, P. H.: Atropine: a sensitive gas chromatography: mass spectrometry assay and prepharmacokinetic studies. Agents Actions **11:** 520–531, 1981.
EHRNEBO, M.: Influence of drug binding to blood cells on pharmacokinetics. Acta Pharm. Suec. **17:** 81–82, 1980.
EHRNEBO, M., AND ODAR-CEDERLÖF, I.: Binding of amobarbital, pentobarbital and diphenylhydantoin to blood cells and plasma proteins in healthy volunteers and uremic patients. Eur. J. Clin. Pharmacol. **8:** 445–453, 1975.
EHRNEBO, M., AND ODAR-CEDERLÖF, I.: Distribution of pentobarbital and diphenylhydantoin between plasma and cells in blood: effect of salicylic acid, temperature and total drug concentration. Eur. J. Clin. Pharmacol. **11:** 37–42, 1977.
ELLION, G. B., AND HITCHINGS, G. H.: Azathioprine. *In* Handbook of Experimental Pharmacology II, ed. by A. C. Sartorelli and D. G. Johns, vol. 38, pp. 404–425, Springer-Verlag, Berlin, 1975.
FASANMADE, A. A., AND JUSKO, W. J.: An improved pharmacodynamic model for formation of methemoglobin by antimalarial drugs. Drug Metab. Dispos. **23:** 573–576, 1995.
FERRARI, V., AND CUTLER, D. J.: Uptake of chloroquine by human erythrocytes. Biochem. Pharmacol. **39:** 753–762, 1990.
FICHTL, B., AND KURZ, H.: Binding of drugs to human muscle. Eur. J. Clin. Pharmacol. **14:** 335–340, 1978.
FLEUREN, H. L. J., AND VAN ROSSUM, J. M.: Nonlinear relationship between plasma and red blood cell pharmacokinetis of chlorthalidone in man. J. Pharmacokinet. Biopharm. **5:** 359–375, 1977.
FREMSTAD, D., AND BERGERUD, K.: Plasma protein binding of drugs as influenced by blood collection methods. Acta Pharmacol. Toxicol. **39:** 570–572, 1976.
FUNDER, J., AND WIETH, O. J.: Chloride and hydrogen ion distribution between human red cells and plasma. Acta Physiol. Scand. **68:** 234–249, 1966.
GAMBHIR, K. K., NERURKAR, S. G., DAS, D. P., ARCHER, J. A., AND HENRY, W. L.: Insulin binding and degradation by human erythrocytes at physiological temperature. Endocrinology **109:** 1787–1789, 1981.
GARAY, R., SENN, N., AND OLLIVER, J. P.: Erythrocyte ion transport as indicator of sensitivity to antihypertensive drugs. Am. J. Med. Sci. **307** (Supplement 1): S120–125, 1994.
GARRETT, E. R., AND HUNT, C. A.: Physicochemical properties, solubility, and protein binding of Δ^g-tetrahydrocannabinol. J. Pharm. Sci. **63:** 1056–1064, 1974.
GARRETT, E. R., ROOSEBOOM, H., GREEN, J. R., AND SCHUERMANN, W.: Pharmacokinetics of papaverin hydrochloride and the biopharmaceutics of its oral dosage forms. Int. J. Clin. Pharmacol. **16:** 193–203, 1978.
GARVER, D. L., DEKIRMENJIAN, H., DAVIS, J. M., CASPER, R., AND ERICKSEN, S.: Neuroleptic drug levels and therapeutic response: preliminary observations with red blood cells - bound butaperazine. Am. J. Psychiatry **134:** 304–307, 1977.
GIEBEL, P., AND PASSOW, H.: Die Permeabilität der Erythrocyten Membran für organische Anionen. Pfluegers Arch. **271:** 378–388, 1960.
GORCZYNSKI, R.: Basic pharmacology of esmolol. Am. J. Cardiol. **56:** 3F–13F, 1985.
GORESKY, C. A.: Linear model for determining liver sinuisoidal and extravascular volumes. Am. J. Physiol. **204:** 626–640, 1963.
GORESKY, C. A., BACH, G. G., AND NADEAU, B.: Red cell carriage of label. Circ. Res. **36:** 328–351, 1975.
GRATZER, W. B.: The red cell membrane and its cytoskeleton. Biochem. J. **198:** 1–8, 1981.
GROSSMAN, S. J., AND JOLLOW, D. J.: Role of dapsone hydroxylamine in dapsone induced hemolytic anemia. J. Pharmacol. Exp. Ther. **244:** 118–125, 1988.
HAMBERGER, C., URIEN, S., BARRE, J., BRANDEBOURGER, M., LEMAIRE, M., LANG, P. H., BUISSON, C., LOISANCE, D., CACHERA, J. P., LAGRUE, G., AND TILLEMENT, J. P.: Distribution of cyclosporin A between blood cells and plasma of cardiac and renal transplant recipients. Ther. Drug Monit. **10:** 28–33, 1988.
HARRIS, P. A., AND RIEGELMAN, S.: Acetylsalicylic acid hydrolysis in human blood and plasma I. J. Pharm. Sci. **56:** 713–716, 1967.
HASEGAWA, T., HARA, K., AND HATA, S.: Binding of dorzolamide and its metabolite, N-deethylated dorzolamide, to human erythrocytes in vitro. Drug Metab. Dispos. **22:** 377–382, 1996.

HEBDEN, H. F., HADFIELD, J. R., AND BEER, C. T.: The binding of vinblastine by platelets in the rat. Cancer Res. **30:** 1417–1423, 1970.
HERNGREN, L., EHRNEBO, M., AND BORÉUS, L. O.: Drug distribution in whole blood of mothers and their newborn infants. Studies of cloxacillin and flucloxacillin. Eur. J. Clin. Pharmacol. **22:** 351–358, 1982.
HILZENBECHER, C.: Die Bindung von Pharmaka an Humanhaemoglobin. Dissertation, Medical School, Ludwig Maximilian University, Munich **1972:** .
HINDERLING, P. H., BRÈS, J., AND GARRETT, E.: Protein binding and erythrocyte partitioning of disopyramide and its monodealkylated metabolite. J. Pharm. Sci. **63:** 1684–1690, 1974.
HINDERLING, P. H.: Kinetics of partitioning and binding of digoxin and its analogues in the subcompartments of blood. J. Pharm. Sci. **73:** 1042–1053, 1984.
HINDERLING, P. H., SCHMIDLIN, O., AND SEYDEL, J. K.: Quantitative relationships between structure and pharmacokinetics of beta-adrenoceptor blocking agents in man. J. Pharmacokinet. Biopharm. **12:** 263–287 and 659–666, 1984.
HINDERLING, P. H.: Comparative evaluation of equilibrium dialysis methods employing biological and artificial membranes for the determination of protein binding of drugs. Ther. Drug Monit. **9:** 331–336, 1987.
HINDERLING, P. H.: Drug distribution in the body: in vitro prediction and physiological interpretation. Prog Pharmacol **6:** 1–30, 1988.
HO NCOC-TA TRUNG, A., SIROIS, G., DUBÉ, M. L., AND MCGILVERAY, I. J.: Comparison of the erythrocyte partitioning method with two classical methods estimating free fraction in plasma. Biopharm. Drug Dispos. **5:** 281–290, 1984.
HOFFMAN, J. F., AND LARIS, P. C.: Determination of membrane potential in human and amphiuma red blood cells by means of a fluorescent probe. J. Physiol. **239:** 519–552, 1974.
HOLDER, L. B., AND HAYES, S. I.: Diffusion of sulfonamides in aqueous buffers into red cells. Mol. Pharmacol. **1:** 266–279, 1965.
HOLMBERG, L., ODAR-CEDERLÖF, I., NILSSON, J. L. G., EHRNEBO, M., AND BORÉUS, L. O.: Pethidine binding to blood cells and plasma proteins in old and young subjects. Eur. J. Clin. Pharmacol. **23:** 457–461, 1982.
HOOKS, M. A.: Tacrolimus, a new immunosuppressant - a review of the literature. Ann. Pharmacother. **28:** 501–511, 1994.
HUFFMAN, D., AND BACHUR, N. R.: Daunorubicin metabolism by human hematological components. Cancer Res. **32:** 600–605, 1972.
HUTABARAT, R., SMITH, A. L., AND UNADKAT, J. D.: Disposition of drugs in cystic fibrosis VII: acetylation of sulfamethoxazole in blood cells: in vitro-in vivo correlation and characterization of its kinetics of acetylation in lymphocytes. Clin. Pharmacol. Ther. **55:** 427–433, 1994.
INABA, T., KALOW, W., SOMEYA, T., TAKAHASHI, S., CHEUNG, S. W., AND TANG, S. W.: Haloperidol reduction can be assayed in human red cells. Can. J. Physiol. Pharmacol. **67:** 1468–1469, 1989.
INGS, R. M. J., NEUDEMBERG, F., BURROWS, J. L., AND BRYCE, T. A.: The pharmacokinetics of oxypentifyllin in man when administered by constant infusion. Eur. J. Clin. Pharmacol. **23:** 539–543, 1992.
JACOBSOHN, G., SIEGEL, E. T., AND JACOBSOHN, M. K.: 17β-Estradiol transport and metabolism in human red blood cells. J. Clin. Endocrinol. Metab. **40:** 177–185, 1975.
JIA, L., BONAVENTURA, C., BONAVENTURA, T., AND STAMLER, S. S: S-nitrosohaemoglobin: a dynamic activity of blood involved in vascular control. Nature (Lond.) **380:** 221–226, 1996.
JUN, H. W., AND LEE, B. H.: Distribution of tetracycline in red blood cells. J. Pharm. Sci. **65:** 455–457, 1980.
JUSKO, W. J., AND D'AMBROSIO, R.: Monitoring FK 506 concentrations in plasma and whole blood. Transplant. Proc. **23:** 2732–2735, 1991.
JUSKO, W. J., PIEKOSZEWSKI, W., KLINTMALM, G. B., SHAEFER, M. S., HEBERT, M. F., PIERGIES, A. A., LEE, C., SCHECHTER, P., AND MEKKI, Q. A.: Pharmacokinetics of tacrolimus in liver transplant patients. Clin. Pharmacol. Ther. **57:** 281–290, 1995.
KAWAI, R., LEMAIRE, M., STEIMER, J. L., BRUELISAUER, A., NIEDERBERGER, W., AND ROWLAND, M.: Physiologically based pharmacokinetic study on a cyclosporin derivative, SDZ IMM 125. J. Pharmacokinet. Biopharm. **22:** 327–365, 1994.
KAWAI, S., OGAWA, K., AND SATAKE, T.: Erythrocyte digoxin concentrations. Clin. Pharmacol. Ther. **31:** 541–547, 1982.
KEIDAN, A. J., SOWTER, M. C., JOHNSON, C. S., MARWAH, S. S., AND STUART, J.: Pharmacological modification of oxygen affinity improves deformability of deoxygenated sickle erythrocytes: a possible therapeutic approach to sickle cell disease. Clin. Sci. **76:** 357–362, 1989.
KEITH, R. A., JARDINE, I., KERREMANS, A., AND WEINSHILBOUM, R. M.: Human erythrocyte membrane thiolmethyltransferase. Drug Metab. Dispos. **12:** 717–724, 1984.
KEITH, R. A., OTTERNESS, D. M., KERREMANS, A. L., AND WEINSHILBOUM, R. M.: S-Methylation of D- and L-penicillamine by human erythrocyte membrane thiol methyltranferase. Drug Metab. Dispos. **83:** 669–676, 1985.
KINTER, W. B., AND PAPPENHEIMER, J.: Renal extraction of PAH and diodrast as a function of arterial red cell concentration. Am. J. Physiol. **185:** 391–398, 1956a.
KINTER, W. B., AND PAPPENHEIMER, J.: Role of red blood corpuscules in regulation of renal blood flow and glomerular filtration rate. Am. J. Physiol. **185:** 399–405, 1956b.
KLOTZ, I. M., AND TAM, J. W. O.: Acetylation of sickle cell hemoglobin by aspirin. Proc. Natl. Acad. Sci. USA **70:** 1313–1315, 1973.
KORNGUTH, M. L., AND KUNIN, C. M.: Uptake of antibiotics by human erthrocytes. J. Infect. Dis. **133:** 175–184, 1976.
KRAMER, K., THURAU, K., AND DEETJEN, P.: Hämodynamik des Nierenmarks I. Mitteilung. Capillaere Passagezeit, Blutvolumen, Durchblutung, Gewebshämatokrit und O_2-Verbrauch des Nierenmarks in situ. Pfluegers Arch. Ges. Physiol. **270:** 251–269, 1960.
KURATA, D., AND WILKINSON, G. D.: Erythrocyte uptake and plasma binding of diphenylhydantoin. Clin. Pharmacol. Ther. **16:** 355–362, 1974.
KUROKAWA, N., KADOBAYASHI, M., YAMAMOTO, K., ARAKAWA, Y., SAWADA, M., TAKAHARA, S., OKUYAMA, A., AND YANAIHARA, C.: In-vivo distribution and erythrocyte binding characteristics of cyclosporin in renal transplant patients. J. Pharm. Pharmacol. **48:** 553–559, 1996.
KURZ, M., AND FICHTL, B.: Binding of drugs to tissues. Drug Metab. Rev. **14:** 467–510, 1983.
KUUSISTO, J., MYKKÄNEN, L., PYÖRÄLÄ, K., AND LAASKO, M.: NIDDM and its metabolic control predict coronary heart disease in elderly subjects. Diabetes **43:** 960–967, 1994.
KWANT, W. O., AND SEEMAN, P.: The membrane concentration of a local anesthetic (chlorpromazine). Biochim. Biophys. Acta **483:** 530–543, 1969.
LAUF, P. K., BAUER, J., ADRAGNA, N., FUJISE, H., ZADE-OPPEN, A. M. M., RYU, K. H., AND DELPIRE, F.: Erythrocyte K-Cl cotransport: properties and regulation. Am. J. Physiol. **263:** C917–C932, 1992.
LEE, C., MARBURY, T., BENET, L. Z.: Clearance calculations in hemodialysis: application to blood, plasma, and dialysate measurements for ethambutol. J. Pharmacokinet. Biopharm. **8:** 69–81, 1980.
LEE, M. G., CHEN, M. L., AND CHIOU, W. L.: Pharmacokinetics of drugs in blood: II—unusual distribution and storage effect of furosemide. Res. Commun. Chem. Pathol. Pharmacol. **34:** 17–28, 1981a.
LEE, M. G., CHEN, M. L., HUANG, S. M., AND CHIOU, W. L.: Pharmacokinetics of drugs in blood: I—unusual distribution of gentamycin. Biopharm. Drug Dispos. **2:** 89–97, 1981b.
LEE, H-J., AND CHIOU, W. L.: Erythrocytes as barriers for drug elimination in the isolated rat liver: I—doxorubicin. Pharm. Res. **6:** 833–839, 1989a.
LEE, H-J., AND CHIOU, W. L.: Erythrocytes as barriers for drug elimination in the isolated rat liver: II—propranolol. Pharm. Res. **6:** 840–843, 1989b.
LEE, M. G., LUI, C. Y., CHEN, M. L., AND CHIOU, W. L.: Pharmacokinetics in blood: IV—unusual distribution, storage effects and metabolism of methotrexate. Int. J. Clin. Pharmacol. Ther. Toxicol. **22:** 530–537, 1984.
LEE, M. G., LUI, C. Y., AND CHIOU, W. L.: Pharmacokinetics of drugs in blood V: Aberrant blood and plasma concentration profiles of methotrexate during intravenous infusion. Biopharm. Drug Dispos. **7:** 487–494, 1986.
LEINWEBER, F. J., AND DICARLO, F. J.: Bunolol metabolism by human and rat red blood cells and extrahepatic tissues. J. Pharmacol. Exp. Ther. **189:** 271–277, 1974.
LENNARD, L.: The clinical pharmacology of 6-mercaptopurine. Eur. J. Clin. Pharmacol. **43:** 329–339, 1992.
LENNARD, L., LILLEYMAN, J. S., VAN LOON, J., AND WEINSHILBOUM, R. M.: Genetic variation in response to 6-mercaptopurine for childhood acute lymphoplastic leukaemia. Lancet **336:** 225–229, 1990.
LENNARD, L., AND MADDOCKS, J. L.: Assay of 6-thioguanine nucleotide, a major metabolite of azathioprine, 6-mercaptopurine and 6-thioguanine, in human red cells. J. Pharm. Pharmacol. **35:** 15–18, 1983.
LIN, J. H., LIN, T. H., AND CHENG, H.: Uptake and stereoselective binding of enantiomers of MK-927, a potent carbonic anhydrase inhibitor, by human erythrocytes in vitro. Pharm. Res. **9:** 339–344, 1992.
LOO, T. T., LU, K., AND SAVARAJ, N.: Erythrocyte as an extrahepatic drug metabolizing tissue. Pharm. Res. **2:** 1645, 1987 (abstract).
LUZZATTO, L.: About hemoglobins, G-6-PD and parasites in red cells. Experientia **51:** 206–208, 1995.
MANDELBAUM-SHAVIT, F., AND BLONDHEIM, S. H.: Acetylation of p-aminobenzoic acid by human blood. Biochem. Pharmacol. **30:** 65–69, 1981.
MÄNNL, H. F., AND HEMPEL, K.: Catechol-O-methyl transferase in human erythrocytes. Naunyn-Schmiedeberg's Arch. Pharmacol. **272:** 265–276, 1972.
MAREN, T. H.: Carbonic anhydrase: chemistry, physiology, and inhibition. Physiol. Rev. **7:** 595–781, 1967.
MASSIL, S. E., SHI, G-Y., AND KLOTZ, I. M.: Acylation of hemoglobin by aspirin-like diacyl esters. J. Pharm. Sci. **73:** 1013–1014, 1984.
MATSUMOTO, Y., ONSAKO, M., AND NODA, F.: Transport of drugs through human erythrocyte membrane in vitro. Yakuzaigaku **1:** 59–63, 1989.
MIDHA, K. K., LOO, J. C. K., AND ROWE, M. L.: The influence of blood sampling technique on the distribution of chlorpromazine and tricyclic antidepressants between plasma and whole blood. Res. Comm. Psychol. Psych. Behav. **4:** 193–203, 1979.
MIGEON, C., LESCURE, O. I., ZINKHAM, W. H., AND SIDBURY, J. B.: In vitro interconversion of 16-C^{14}-estrone and 16-C^{14}-estradiol-17β by erythrocytes from normal subjects and from subjects with a deficiency of red cell glucose-6-phosphate dehydrogenase activity. J. Clin. Invest. **41:** 2025–2035, 1962.
MINAMI, T., AND CUTLER, D. J.: A kinetic study of the role of band 3 anion transport protein in the transport of salicylic acid and other hydroxybenzoic acids across the human erythrocyte membrane. J. Pharm. Sci. **81:** 424–427, 1992.
MOHAMMED, S. S., CHRISTOPHER, M. M., METHA, P., KEDAR, A., GROSS, S., AND

DERENDORF, H.: Increased erythrocyte and protein binding of codeine in patients with sickle cell disease. J. Pharm. Sci. **82:** 1112–1117, 1993.
MORGAN, D. J., GUTTMANN, A., WATSON, P. R. G., GHABRIAL, H., ELLIOTT, S. L., AND SMALLWOOD, R. A.: Effect of erythrocyte binding on elimination of harmol by the isolated perfused rat liver. J. Pharm. Sci. **85:** 40–44, 1996.
MORSCHES, B., BENES, P., HOLZMANN, H., AND HENRICH, B.: Zur Penetration-skinetik von Dehyroepiandrosteron durch die Erythrocytenmembran. Arch. Dermatol. Res. **270:** 49–55, 1981.
MOTULSKI, A. G., AND STEINMANN, L.: Arylamine acetylation in human red cells. J. Clin. Invest. **41:** 1387 (abstract), 1962.
MULDER, E., LAMERS-STAHLHOFEN, G. J. M., AND VANDER MOLEN, H. J.: Isolation and characterization of 17β-hydroxy steroid dehydrogenase from human erythrocytes. Biochem. J. **127:** 649–659, 1972.
NERURKAR, S. G., AND GAMBHIR, K. K.: Insulin degradation by human erythrocyte lysates. Clin. Chem. **27:** 607–609, 1981.
NIEDERBERGER, W., LEMAIRE, M., MAURER, K., NUSSBAUMER, K., AND WAGNER, O.: Distribution and binding of cyclosporin in blood and tissues. Transplant. Proc. **15:** 2419–2421, 1983.
NOEL, M.: Kinetic study of normal and sustained release dosage forms of metformin in normal subjects. Res. Clin. Forum **1:** 33–44, 1979.
NOONAN, P. K., AND BENET, L. Z.: Formation of mono- and dinitrate metabolites of nitroglycerin following incubation with human blood. Int. J. Pharm. (Amst.) **12:** 331–340, 1982.
NOSÁL, R., ERICSSON, O., SJÖQVIST, F., AND DURISOVÁ, M.: Distribution of chloroquine in human blood fractions. Methods Find. Exp. Clin. Pharmacol. **10:** 581–587, 1988.
ODEH, Y. K., WANG, Z, RUO, T. I., WANG, T., FREDERIKSEN, M. C., POSPISIL, P. A., AND ATKINSON, JR., A. J.: Simulataneous analysis of inulin and $^{15}N_2$-urea kinetics in humans. Clin. Pharmacol. Ther. **53:** 419–425, 1993.
ORRINGER, E. P., POWELL, R. J., CROSS, R. E., ROGERS, J. F., WOJCIESZYN, O., PHILLIPS, J. C., REED, J., NG, K-T., AND BERKOWITZ, L. R.: A single-dose pharmacokinetic study of the antisickling agent cetiedil. Clin. Pharmacol. Ther. **39:** 276–281, 1986.
OWEN, J. A., AND NAKATSU, K.: Diacetylmorphine (heroin) hydrolases in blood. Can. J. Physiol. Pharmacol. **61:** 870–875, 1983.
PAGE, T., AND CONNOR, J. D.: The metabolism of ribavirin in erythrocytes and nucleated cells. Int. J. Biochem. **22:** 379–383, 1990.
PAPPENHEIMER, J. R., AND KINTER, W. B.: Hematocrit ratio of blood within mammalian kidney and its significance for renal hemodynamics. Am. J. Physiol. **185:** 377–390, 1956.
PAZMIÑO, P. A., SLADEK, S. L., AND WEINSHILBOUM, R. M.: Thiol S-methylation in uremia: erythrocyte enzyme activities and plasma inhibitors. Clin. Pharmacol. Ther. **28:** 346–367, 1980.
PECK, C. C., BARR, W. H., BENET, L. Z., COLLINS, J., DESJARDINS, R. E., FURST, D. E., HARTER, J. G., LEVY, G., LUDDEN, T., REDMAN, J. H., SANATHANAN, L., SCHENTAG, J. J., SHAH, V. P., SHEINER, L. B., SKELLY, J. P., STANSKI, D. R., TEMPLE, R. J., VISWANATHAN, C. T., WEISSINGER, J., AND YACOBI, A.: Opportunities for integration of pharmacokinetics, pharmacodynamics, and toxicokinetics in rational drug development. Clin. Pharmacol. Ther. **51:** 465–473, 1992.
PIAZZA, E., BROGGINI, M., TRABATTONI, A., NATALE N., LIBRETTI, A., AND DONNELLI, M. G.: Adriamycin distribution in plasma and blood cells of cancer patients with altered hematocrit. Eur. J. Cancer Clin. Oncol. **17:** 1089–1096, 1981.
PIRMOHAMED, M., COLEMAN, M. D., HUSSAIN, F., BRECKENRIDGE, A. M., AND PARK, B. K.: Diet and metabolism dependent toxicity of sulphasalazine and its principal metabolites toward human erythrocytes and leucocytes. Br. J. Clin. Pharmacol. **32:** 303–310, 1991.
QUON, C. Y., AND STAMPFLI, H. F.: Biochemical properties of blood esmolol esterase. Drug Metab. Dispos. **13:** 420–424, 1985.
RAMBO, L., ERICSSON, O., ALVÁN, G., LINDSTROEM, B., GUSTAFSSON, L. L., AND SJÖQVIST, F.: Chloroquin and desethylchloroquine in plasma, serum and whole blood: problems in assay and handling of samples. Ther. Drug Monit. **7:** 211–215, 1985.
RATGE, D., KOHSE, K. P., STEEGMUELLER, U., AND WISSER, H.: Distribution of free and conjugated catecholamines between plasma platelets and erythrocytes: different effects of intravenous and oral catecholamine administrations. J. Pharmacol. Exp. Ther. **257:** 232–238, 1991.
REICHEL, C., VON FALKENHAUSEN, M., BROCKMEIER, D., AND DENGLER, H. J.: Characterization of cyclosponine A uptake in human erythrocytes. Eur. J. Clin. Pharmacol. **46:** 417–419, 1994.
REPKE, K,., AND MARKWARDT, F.: Über fermentative Reduktion von Oestron. Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmakol. **223:** 271–279, 1954.
ROLAN, P. E., PARKER, J. E., GRAY, S. J., WEATHERLEY, B. C., INGRAM, J., LEAVENS, W., WOOTTON, R., AND POSNER, J.: The pharmacokinetics, tolerability and pharmacodynamics of tucaresol (589C80; 4[2-formyl-3-hydroxyphenoxymethyl]benzoic acid), a potential antisickling agent, following oral administration. Br. J. Clin. Pharmacol. **30:** 419–425, 1993.
ROOS, A., AND HINDERLING, P. H.: Protein binding and erythrocyte partitioning of the antirheumatic proquazone. J. Pharm. Sci. **70:** 252–257, 1981.
ROSTAMI-HODJEGAN, A., LENNARD, L., AND LILLEYMAN, J. S.: The accumulation of mercaptopurine metabolites in age fractionated red blood cells. Br. J. Clin. Pharmacol. **40:** 217–222, 1995.
RUDY, A. C., AND POYNOR W. J.: Binding of pyrimethamine to human plasma proteins and erythrocytes. Pharm. Res. **7:** 1055–1060, 1990.
RYLANCE, H. J., AND WALLACE, R. C.: Erythrocyte and plasma aspirin esterase. Br. J. Clin. Pharmacol. **12:** 436–438, 1981.
SAN GEORGE, R. G., NAGEL, R. L., AND FABRY, M. E.: On the mechanism for red cell accumulation of mefloquin, an antimalaria drug. Biochim. Biophys. Acta **803:** 174–181, 1984.
SARTORIS, D. J., GOODWIN, D. A., MEARES, C. F., DERIEMER, L. H., AND FAJARDO, L. F.: Pharmacokinetics of Indium-111 BLEDTA in man. Invest. Radiol. **19:** 221–227, 1984.
SCHAAF, L., DOBBS, B., EDWARDS, R., AND PERRIER, D. G.: Nonlinear pharmacokinetics and whole blood stability of 5-fluorouracil (5-FU) in patients with colorectal cancer. Pharm. Res. **3:** 107S, 1986 (abstract).
SCHANKER, L. S., JOHNSON, J. M., AND JEFFREY, J. J.: Rapid passage of organic anions into human red cells. Am. J. Physiol. **207:** 503–508, 1964.
SCHANKER, L. S., NAFPLIOTIS, P. A., AND JOHNSON, J. M.: Passage of organic bases into human red cells. J. Pharmacol. **133:** 325–331, 1961.
SCHATZMANN, H. J.: Herglycoside als Hemmstoffe fuer den aktiven Kalium- und Natriumtransport durch die Erythrozytenmembran. Helv. Physiol. Pharmacol. Acta **11:** 346–360, 1953.
SCOTT, M. C., VAN LOON, J. A., AND WEINSHILBOUM, R. M.: Pharmacogenetics of N-methylation: heritability of human erythrocyte histamine N-methyltransferase. Clin. Pharmacol. Ther. **43:** 256–262, 1988.
SHA'AFI, R. I., GARY-BOBO, C. M., AND SOLOMON, A. K.: Permeability of red cell membranes to small hydrophilic and lipophilic solutes. J. Gen. Physiol. **58:** 238–258, 1971.
SHIN WAN, G., LEE, M. G., LEE, M. H., KIM, N. D.: Pharmacokinetics of drugs in blood: VII—unusual distribution and blood storage effect of vancomycin. Biopharm. Drug Dispos. **13:** 305–310, 1992.
SHIRKEY, R. J., JELLETT, L. B., KAPPATOS, D. C., MALING, T. J. B., AND MCDONALD, A.: Distribution of sodium valproate in normal whole blood and in blood from patients with renal or hepatic disease. Eur. J. Clin. Pharmacol. **28:** 447–452, 1985.
SKALSKI, M., SCHINDHEIM, K., AND FARRELL, P.: Creatinine transfer between red cells and plasma: a comparison between normal and uremic subjects. Nephron **22:** 514–521, 1978.
SNOEK, E., JACQMIN, P., VAN PEER, E., DANHOF, M., VER DONK, K., VAN BELLE, H., WOESTENBORGHS, R., CRABBÉ, R., VAN GOOL, R., DUPONT, A., AND HEYKANTS, J.: The implications of nonlinear red cell partitioning for the pharmacokinetics and pharmacodynamics of the nucleoside inhibitor draflazine. Br. J. Clin. Pharmacol. **42:** 605–613, 1996.
SOKOLOSKI, T. D., WU, C. C., WU, L. S., AND BURKMAN, A. M.: Interaction of nitroglycerin with human blood components. J. Pharm. Sci. **72:** 335–338, 1983.
SOLOMON, A. K., CHASAN, B., DIX, J. A., LUKACOVIC, M. F., TOON, M. R., AND VERKMAN, A. S.: The aqueous pore in the red cell membrane: band 3 as a channel for anions, cations, nonelectrolytes, and water. Ann. N. Y. Acad. Sci. **414:** 229–234, 1983.
SPECTOR, W. S.: Handbook of Biological Data, pp. 52 and 70, W. B. Saunders, Philadelphia, 1956.
STEC, G. P., AND ATKINSON JR., A. J.: Analysis of the contributions of permeability and flow to intercompartmental clearance. J. Pharmacokinet. Biopharm. **9:** 167–180, 1981.
STEC, G. P., ATKINSON JR., A. J., NEVIN, M. J., THENOT, J. P., RUO, T. I., GIBSON, T. P., IVANOVICH, P.,AND DEL GRECO, F.: N-acetylprocainamide pharmacokinetics in functionally anephric patients before and after perturbation by hemodialysis. Clin. Pharmacol. Ther. **26:** 618–628, 1979.
STONE, P. C. W., NASH, G. B., AND STUART, J.: Substituted benzaldehydes (12C79 and 589C80) that stabilize oxyhemoglobin also protect sickle cells against calcium-mediated dehydration. Br. J. Haematol. **81:** 419–423, 1992.
SVENSSON, C., NYBERG, G., AXELSSON, R., AND MÅRTENSSON, E.: Concentrations of thioridazine and thioridazine metabolites in erythrocytes. Psychopharmacology **89:** 291–293, 1986.
SWEENEY, K. R., CAPRON, D. J., AND KRAMER, P. A.: Effect of salicylate on serum protein binding and red cell uptake of acetazolamide in vitro. J. Pharm. Sci. **77:** 751–756, 1988.
SZUMLANSKI, C. L., HONCHEL, R., SCOTT, M. C., AND WEINSHILBOUM, R. M.: Human liver thiopurine methyltransferase pharmacogenetics: biochemical properties, liver-erythrocyte correlation and presence of isozymes. Pharmacogenetics **2:** 148–159, 1992.
TETT, S. E., CUTLER, D. J., DAY, R. O., AND BROWN, K. F.: A dose-ranging study of the pharmacokinetics of hydroxychloroquine following intravenous administration to healthy volunteers. Br. J. Clin. Pharmacol. **26:** 303–313, 1988.
TRAFICANTE, L. J., SAKALIS, G., SIEKIERSKI, J., ROFROSEN, J., AND GERSHON, S.: Rapid in vitro sulfoxidation of chlorpromazine by human blood: inhibition by an endogenous plasma protein factor. Life Sci. **24:** 337–346, 1979.
TUCKER, G. T., CASEY, C., PHILLIPS, P. J., CONNOR, M., WARD, J. D., AND WOODS, H. F.: Metformin kinetics in healthy subjects and in patients with diabetes mellitus. Br. J. Clin. Pharmacol. **12:** 235–246, 1981.
URIEN, S., BASTIAN, G., LUCAS, C., BIZZARI, J-P., AND TILLEMENT, J. P.: Binding of a new Vinca alkaloid derivative, S12363, to human plasma proteins and platelets: usefulness of an erythrocyte partitioning technique. Invest. New Drugs **10:** 263–268, 1992a.
URIEN, S., CLAUDEPIERRE, P., MEYER, J., BRANDT, R., AND TILLEMENT J. P.: Comparative binding of etretinate and acitretin to plasma proteins and erythrocytes. Biochem. Pharmacol. **44:** 1891–1893, 1992b.

URIEN, S., RIANT, P., RENOUARD, A., COULOMB, B., ROCHER, I., AND TILLEMENT, J. P.: Binding of indapamide to serum proteins and erythrocytes. Biochem. Pharmacol. **37:** 2963–2966, 1988.

VAN BELLE, H.: Nucleoside transport inhibition: a therapeutic approach to cardioprotection via adenosine. Cardiovasc. Res. **27:** 68–76, 1993.

VAN DER MOLEN, H. J., AND GROEN, D.: Interconversion of progesterone and 20α–dihydroprogesterone and of androstenedione and testosterone in vitro by blood and erythrocytes. Acta Endocrinol. **58:** 419–444, 1968.

VAN GRIENSVEN, J. M. T., JUSKO, W., LEMKES, H. H. P. J., KROON, R, VERHORST, C. J., CHIANG, S. T., AND COHEN, A. F.: Tolrestat pharmacokinetic and pharmacodynamic effects on red blood cell sorbitol levels in normal volunteers and in patients with insulin-dependent diabetes. Clin. Pharmacol. Ther. **58:** 631–640, 1995.

VENITZ, J., GERBER, M., AND ABRAHAM, D.: Pharmacological effects of escalating iv doses of an allosteric hemoglobin modifier, RSR13, in healthy volunteers. Pharm. Res. **13:** S-115, 1996 (abstract).

VERDIER, F., LE BRAS, J., CALVIER, F., HATIN, I., AND BLAYO, M. C.: Chloroquine uptake by Plasmodium falciparum-infected human erythrocytes during in vitro culture and its relationship to chloroquine resistance. Antimicrob. Agents Chemother. **27:** 561–564, 1985.

VERONESE, M. E., MCLEAN, S., AND HENDRIKS, R.: Plasma protein binding of amiodarone in a patient population: measurement by erythrocyte partitioning and a novel glass binding method. J. Clin. Pharmacol. **26:** 721–731, 1980.

VIDREQUIN, S., GIMENEZ, F., BASCO, L. K., MARTIN, C., LE BRAS, J., AND FARINOTTI, R.: Uptake of mefloquine enantiomers into uninfected and malaria-infected erythrocytes. Drug Metab. Dispos. **24:** 689–691, 1996.

WALIN, A., AND HERNGREN, L.: Distribution of phenobarbital in whole blood during pregnancy and perinatally: an in vitro study. Eur. J. Clin. Pharmacol. **29:** 187–191, 1985.

WALLACE, S. M., AND RIEGELMAN, S.: Uptake of acetazolamide by human erythrocytes. J. Pharm. Sci. **66:** 729–731, 1977.

WEINSHILBOUM, R. M., AND SLADEK, S. L.: Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltranferase activity. Am. J. Hum. Genet. **32:** 651–662, 1980.

WENK, M., AND FOLLATH, F.: Temperature dependency of apparent cyclosporin A concentrations in plasma. Clin. Chem. **29:** 1865, 1983.

WIERSMA, D. A., STEMMER, P. M., AND ROTH, R. A.: Influence of red blood cells, serum albumin, and serum lipoproteins on the clearance of benzo[a]pyrene by isolated livers of 3-methylcholanthrene-treated rats. Biochem. Pharmacol. **33:** 3433–3438, 1984.

WILKINSON, G. R.: Clearance approaches in pharmacology. Pharmacol. Rev. **39:** 1–47, 1987.

WIND, M., BERLINER, A., AND STERN, S.: The binding of phenothiazines to oxyhemoglobin A and S. Res. Commun. Chem. Pathol. Pharmacol. **5:** 759–766, 1973.

WINSTANLEY, P., EDWARDS, G., ORME, M., AND BRECKENRIDGE, A.: The disposition of amodiaquine in man after oral administration. Br. J. Clin. Pharmacol. **23:** 1–7, 1987.

WOODSON, L. C., DUNNET, J. H., AND WEINSHILBOUM, R. M.: Pharmacogenetics of human thiopurine methyltransferase: kidney erythrocyte correlation and immunotitration studies. J. Pharmacol. Exp. Ther. **222:** 174–181, 1982.

YATSCOFF, R. W., HONCHARIK, N., LUKOWSKI, M., THLIVERIS, J., CHACKOWSKI, P., AND FARACI, C.: Distribution of cyclosporin G (NVA^2 cyclosporin) in blood and plasma. Clin. Chem. **39:** 213–217, 1993a.

YATSCOFF, R. W., AND JEFFREY, R. J.: Effects of sample preparation on cyclosporin G (NVA^2-cyclosporine) concentration. Clin. Chem. **33:** 1257–1260, 1987.

YATSCOFF, R. W., LEGATT, D., KEENAN, R., AND CHACKOWSKI, P.: Blood distribution of rapamycin. Transplantation **56:** 1202–1206, 1993b.

YAYON, A., AND GINSBURG, H.: The transport of chloroquine across erythrocyte membranes is mediated by a simple symmetric carrier. Biochim. Biophys. Acta **686:** 197–203, 1982.

ZAUGG, R. H., WALDER, J. A., WALDER, R. Y., STEELE, J. M., AND KLOTZ, I. M.: Modification of hemoglobin with analogs of aspirin. J. Biol. Chem. **285:** 2816–2821, 1980.

ZIMMERMAN, T. P., AND DEEPROSE, R. D.: Metabolism of 5-amino-1-D-ribofuranosyl-imidazole-4-carboxamide and related five-membered heterocycles to 5′-triphosphates in human blood and L 5178 cells. Biochem. Pharmacol. **27:** 709–716, 1978.