Distribution of Tetracycline in Red Blood Cells

H. W. JUN ˣ and B. H. LEE

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Abstract □ The distribution of tetracycline into human red blood cells was studied *in vitro* at 37°. The drug was taken up rapidly by the cells, and its distribution equilibrium was reached after ~10 min of incubation. The steady-state distribution ratio of the drug between the red cells and extracellular fluid of 0.9% NaCl solution was 2.84, while the distribution ratio between the cells and the plasma solution was 0.9. The reduced cellular uptake in plasma was due to binding of the drug to plasma components. Calcium ions in plasma appeared to reduce tetracycline uptake by the red cells. The red cell distribution of tetracycline in dogs was in close agreement with the *in vitro* data using the washed human red cells. A dog suffering from severe hypoalbuminemia showed greater uptake of the drug by the erythrocytes, while a normal healthy dog exhibited the red cell–plasma distribution ratio of 0.98. The release rate of tetracycline from the preloaded cells into normal saline solution indicated that the release was directly proportional to the cellular concentration of the drug; the first-order release rate was ~1.38 hr^{-1}.

Keyphrases □ Tetracycline—distribution in red blood cells □ Antibacterials—tetracycline, distribution in red blood cells □ Drug distribution—tetracycline, uptake by red blood cells

One important function of red blood cells is to store hemoglobin, which carries and supplies oxygen to various tissues in the body. Drugs can enter red blood cells and bind to the intracellular constituents (1–3). For many drugs, however, erythrocyte uptake means that less drug is available at the site of pharmacological action.

Plasma and serum concentrations have been monitored commonly to study the pharmacokinetics of drugs in the body, with the assumption that drug levels in the plasma and serum are linearly related to drug concentrations in whole blood. Although several drugs such as chlorthalidone (4) and salicylate (5) were shown to accumulate significantly in the red cells, several reports (6–8) indicated that extensive plasma protein binding lowers the red cell uptake of the drug by reducing the free plasma drug concentration. In patients suffering from hypoalbuminemia, the basic pharmacokinetic parameters of the drug thus can be different from those obtained in normal patients (9, 10).

This study was an attempt to elucidate the *in vitro* and *in vivo* distribution characteristics of tetracycline into red blood cells and to measure the effects of the normal plasma components on the red cell uptake of this drug.

EXPERIMENTAL

Materials and Equipment—Tetracycline hydrochloride[1], pentobarbital sodium[2], trichloroacetic acid[3], calcium chloride[3], and human serum albumin[2] were used as received. A rotating apparatus[4] with multiple sample holders was immersed in a water bath, which was kept at 37 ± 1° by a controlled-temperature circulating pump[5]. Fluorescence measurements were made using a spectrophotofluorometer[6] equipped with a 150-w xenon lamp and a R-447 photomultiplier tube.

Preparation of Red Cell Suspensions for *In Vitro* Distribution Studies—Human blood, obtained from either healthy young volunteers or a local blood bank[7], was centrifuged at 1200×*g* for 8 min. The packed red cells were obtained after removing the plasma water and buffy layer containing leukocytes. The cells then were washed three times with isotonic buffer solution (pH 7.4). After the final washing, the cells were suspended in either 0.9% NaCl solution or human plasma solution containing varying amounts of tetracycline (5, 10, 20, 30, and 50 μg/ml). These concentrations are slightly higher than normal clinical concentrations of the drug in patients (11).

Distribution of Tetracycline into Cells—The distribution of tetracycline into the red cells was measured by assaying the drug both in the extracellular fluid and in the cells after incubating the samples consisting of 5 ml of washed cells and 5 ml of 0.9% NaCl solution or human plasma containing 20 μg of tetracycline/ml in a rotating water bath (40 rpm) at 37 ± 1°. Following the incubation for different time intervals (3, 5, 10, 15, 20, and 30 min), the cells were separated by centrifugation at 1200×*g* for 8 min for the assay of the drug in the cells and in the medium.

Effect of pH on Cellular Uptake of Tetracycline—Five milliliters of red cells was suspended in 5 ml of phosphate buffer solution (pH 6.7, 7.4, or 8.0) containing 20 μg of tetracycline/ml. After incubation for 15 min in a rotating water bath at 37 ± 1°, the cells were separated by centrifugation. The concentration ratio of tetracycline between the medium and red cells was determined for each pH solution.

Drug Release Rate from Red Cells—The release rate of tetracycline from the preloaded cells into 0.9% NaCl solution was measured by assaying the drug in the extracellular fluid after incubation of the samples in a rotating water bath at 37 ± 1°. Initially, 5 ml of washed red cells, preequilibrated with 5 ml of tetracycline solution (20 μg/ml) in 0.9% NaCl solution for 15 min, was mixed with 5 ml of 0.9% NaCl solution; this mixture was subjected to incubation in a rotating water bath at 37 ± 1° for 10 min. After centrifugation at 1200×*g* for 8 min, the tetracycline concentration in the extracellular medium was measured. The remaining cells then were reequilibrated with an equal volume of fresh 0.9% NaCl solution for 10 min, followed by centrifugation and assay of the drug in the extracellular fluid. The procedure was repeated five times.

Effect of Plasma Albumin and Calcium Ion on Red Cell Uptake of Tetracycline—The cellular uptake of tetracycline was measured in the solutions containing plasma albumin (4%) and/or calcium chloride (2.5×10^{-5} *M*). The results then were compared with the distribution of tetracycline between the red cells and 0.9% NaCl solution.

Red Cell Uptake of Tetracycline in Dogs—*In vivo* distribution of tetracycline between the plasma and red cells was determined in a healthy beagle dog (Dog A) and in another beagle dog (Dog B) suffering from hypoalbuminemic conditions. The red cell counts for Dogs A and B were 5.59×10^6 and 5.48×10^6/ml, respectively. The normal ranges of the average red cell counts and albumin levels in dogs are 5.26×10^6–6.57×10^6/ml and 3.1–4.0%, respectively. The dogs received a bolus intravenous injection of 10 mg of tetracycline/kg, and the blood samples taken at different time intervals were assayed for the drug both in the cells and in the plasma.

Assay of Tetracycline in Cells and Extracellular Fluids—A spectrophotofluorometric method (12) was used. This method measures

[1] American Cyanamid Co., Pearl River, N.Y.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] J. T. Baker Chemical Co., Phillipsburg, N.J.
[4] Menold, Lester, Pa.
[5] Haake E52, Berlin-Lichterfied, West Germany.
[6] Model MPF-4, Perkin-Elmer Corp., Norwalk, Conn.
[7] St. Mary's Hospital, Athens, Ga.

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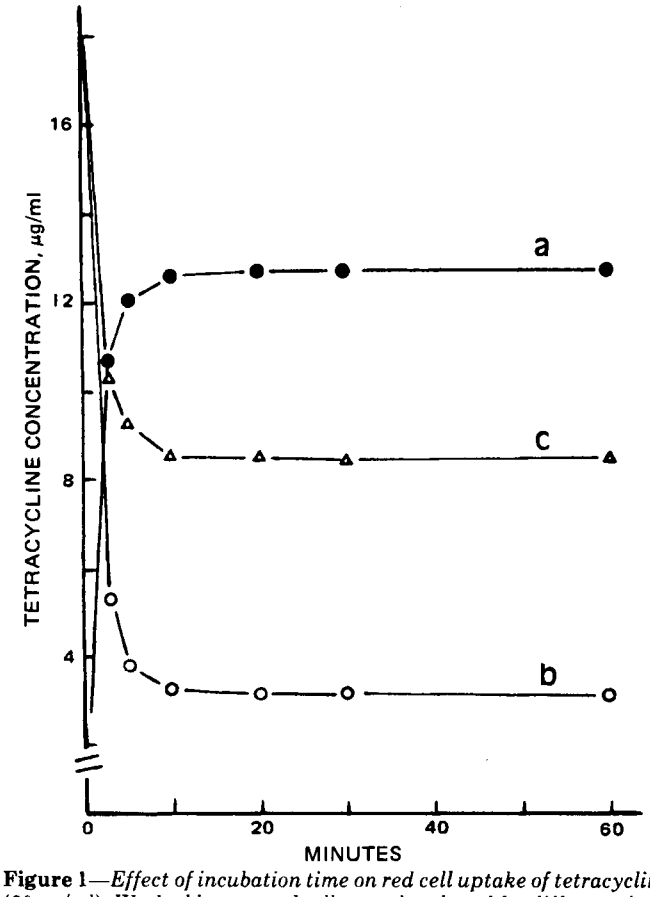

Figure 1—*Effect of incubation time on red cell uptake of tetracycline (20 μg/ml). Washed human red cells were incubated for different times in 0.9% NaCl solution (a, drug in red cells; and b, drug in extracellular fluid) and in human plasma (c, drug in extracellular fluid).*

the fluorescence intensity of the tetracycline–pentobarbital–calcium complex at 525 nm when it is activated at 390 nm.

RESULTS AND DISCUSSION

Figure 1 shows the distribution profile of tetracycline into the red cells as a function of time during the incubation of the washed cells in an equal volume of 0.9% NaCl solution containing 20 μg of tetracycline/ml. During the first 10 min of incubation, the drug was taken up rapidly by the cells, and the steady-state distribution ratio between the red cells and extracellular fluid was ~2.84. The large distribution into the cells probably was due to the significant binding of tetracycline to the intracellular components. However, when tetracycline was subjected to equilibration between the human plasma and erythrocytes, the distribution ratio between the red cells and plasma was 0.9 ± 0.12 (Fig. 1, curve c).

Since plasma contains various proteins and also divalent cations that form lipid-insoluble complexes with tetracycline (13), studies were done to identify the plasma components responsible for the lowered cellular uptake of the drug in the plasma as compared with that in 0.9% NaCl solution. Figure 2 compares the distribution ratios of tetracycline between the red cells and 0.9% NaCl solution (bar A) in the presence of various additives. When both calcium (2.5×10^{-5} *M*) and serum albumin (4%) were added to 0.9% NaCl solution, the steady-state distribution ratio of

Table I—Effect of pH on Steady-State Tetracycline Concentrations in Red Cells and Extracellular Fluid at 37° [a]

	Measured Tetracycline Concentration[b], μg/ml		
	pH 6.7	pH 7.4	pH 8.0
Red blood cells	14.78	14.72	14.79
Medium	5.14	5.24	5.27
Red cell-medium ratio	2.88	2.81	2.81

[a] Initial extracellular concentration was 20 μg/ml. [b] Average of three experiments.

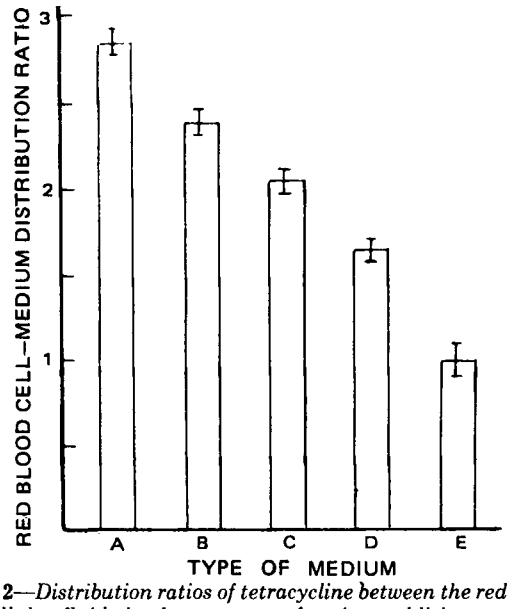

Figure 2—*Distribution ratios of tetracycline between the red cells and extracellular fluids in the presence of various additives at 37°. The extracellular fluids were 0.9% NaCl solution containing 20 μg of tetracycline/ml (A); 20 μg of tetracycline/ml and 2.5×10^{-5} M $CaCl_2$ (B); 20 μg of tetracycline/ml and 4% albumin (C); and 20 μg of tetracycline/ml, 2.5×10^{-5} M $CaCl_2$, and 4% albumin (D); and human plasma containing 20 μg of tetracycline/ml (E). The vertical bars* (I) *represent the standard error of the mean.*

tetracycline between the cells and medium was significantly lower (bar D) than that in the solution containing calcium (bar B) or albumin (bar C) alone. However, the cellular uptake of tetracycline in human plasma (bar E) was still lower than that in 0.9% NaCl solution containing calcium and albumin (bar D).

These results indicate that the components other than calcium and albumin in the plasma also were responsible for the lowered drug partitioning into the cells. Globulins in the plasma possibly may provide additional binding sites to tetracycline. Nevertheless, the distribution of tetracycline into the red cells observed in this study was somewhat similar to the rate of uptake of tetracycline by *Escherichia coli* cells. Franklin and Higginson (14) found that tetracycline rapidly entered the bacterial cells, and its distribution equilibrium between the cells and extracellular medium was reached within 10 min of incubation.

Figure 3 illustrates the effect of tetracycline concentration on the red cell uptake in 0.9% NaCl solution. The steady-state distribution ratio between the cells and extracellular fluid generally was the same among the different total tetracycline concentrations studied. When the concentration of tetracycline was increased from 5 to 50 μg/ml, the distribution of the drug into the cells also was increased while the steady-state distribution ratio between the red cell and the medium was maintained

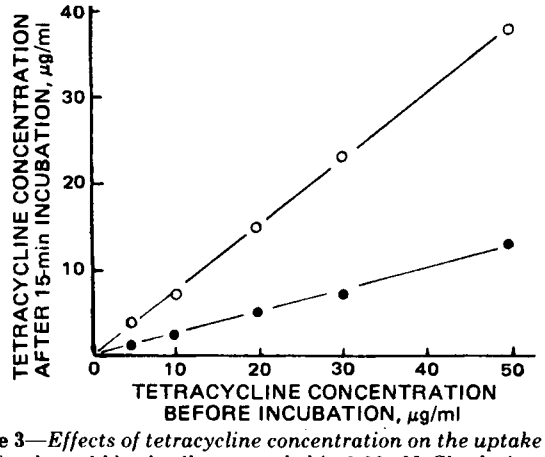

Figure 3—*Effects of tetracycline concentration on the uptake of tetracycline by red blood cells suspended in 0.9% NaCl solution at 37°. Key:* O, *red blood cells; and* ●, *0.9% NaCl solution.*

Table II—Distribution of Tetracycline into Red Blood Cells in Two Dogs

	Dog A	Dog B[a]	
Body weight, kg	24	16.5	16.6
Intravenous dose, mg/kg	10	10	10
Total protein, %	6.81	5.32	5.54
Albumin, %	2.98	1.52	1.84
Hematocrit, %	44.5	37.4	36.9
Red cell–plasma ratio	0.98	1.97	1.74

[a] The data were obtained 2 weeks apart.

at 2.84. This finding probably was due to the fact that a large amount of tetracycline could partition into the cellular components of the red cells.

Table I shows the effect of pH on the distribution of tetracycline into the red cells. Between pH 6.7 and 8.0, the steady-state distribution ratio of the drug remained nearly the same. Since tetracycline undergoes extensive ionization in this pH range, the transport of this drug across cellular membranes probably was mediated by facilitated diffusion as suggested previously (15).

Figure 4 shows the steady-state concentrations of tetracycline in the red cells and the medium after each successive 10-min equilibration with 0.9% NaCl solution. The rate of drug release apparently was an exponential function of the drug concentration in the cells, and the concentration-dependent release rate was $\sim 1.38\ hr^{-1}$. The large release rate constant of the drug from the red cells as compared to its relatively slow

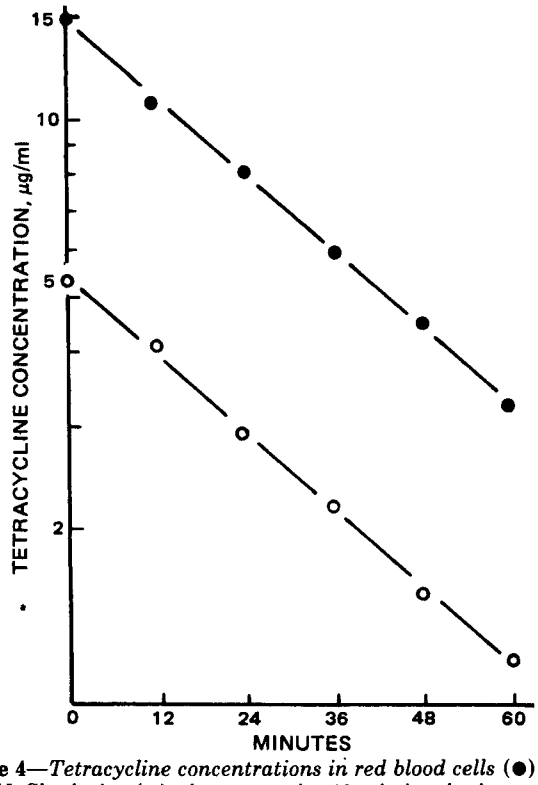

Figure 4—*Tetracycline concentrations in red blood cells (●) and in 0.9% NaCl solution (○) after successive 10-min incubations.*

elimination rate constant ($0.07\ hr^{-1}$) in humans (11) indicates that elimination of tetracycline from the body usually was not affected by the red cell uptake of the drug. Tetracycline will be released quickly from the erythrocytes as the drug in the blood disappears from the body by elimination.

It is generally recognized that the pharmacokinetic behavior of the drug in the normal healthy population may differ from that in patients suffering from chronic illness. Several investigators (9, 10, 16) showed that hypoalbuminemic patients tend to suffer more often than normal patients from adverse drug reactions. Table II compares the distribution of tetracycline in the blood of a dog suffering from severe hypoalbuminemia (1.68%) to that of a normal healthy dog. Dog A, with a normal albumin level (2.98%), had a cell–plasma distribution ratio of 0.98; Dog B, which suffered from hypoalbuminemia, showed a distribution ratio of 1.8, which was nearly two times higher than that in Dog A. These findings are in close agreement with the results obtained from the *in vitro* experiments using the human plasma and the solutions containing different additives.

In summary, tetracycline rapidly enters red blood cells, forming a steady-state distribution equilibrium with the extracellular fluid within 10 min of incubation. The steady-state distribution ratio of the drug between the red cells and extracellular fluid of 0.9% NaCl solution was 2.84, while the distribution ratio between the cells and the plasma solution was 0.9. The low cellular uptake in the plasma was due to binding of the drug to the plasma components. A dog suffering from severe hypoalbuminemia exhibited much greater red cell uptake of tetracycline. The large release rate of the drug from the cells found in this study indicates that the elimination rate of tetracycline from the body is not influenced by the cellular uptake of the drug.

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