Stoichiometry of Compounds Bound to Human Erythrocytes in Relation to Morphology

(Received for publication, May 31, 1974)

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SUMMARY

Most work on human erythrocyte interaction with drugs and other compounds has been reported on the basis of total concentrations. Total concentrations alone do not reveal numbers of molecules bound per cell, v. This paper emphasizes determination of v and of binding isotherms, in conjunction with changes in cell morphologies and in hypotonic shock behavior as v is varied. Four drugs and five other compounds were studied, with fresh erythrocytes.

The principal findings are: (1) the intact erythrocyte engages in two kinds of binding mechanisms, statistical binding and cooperative binding, depending on the compound. In the case of a detergent, dodecylbenzene sulfonate, the binding is nearly quantitative. (2) The compounds often induce considerable protection against hypotonic hemolysis. However, the binding levels at which maximum protection occurs are rather close to the levels, vL, that occur upon complete conversion to the first distorted morphology. Therefore, the maximally protected erythrocyte may be a distorted erythrocyte. (3) The value n is the apparent total number of sites from Scatchard plotting for compounds which bind in a statistical manner. Levels vF and vn characterize maxima in cooperative binding behavior, also from Scatchard plotting of the data. Despite the wide diversity of over-all levels at which compounds exert their effects, the critical binding levels and numbers of sites fall into a narrow range: n, vF, vP, and vn are all between 1 and 8 × 10^6 molecules or sites per cell. Most of our data, and that from some other laboratories, indicate that about 2 ± 1 × 10^6 sites per erythrocyte are available for compound binding by the intact cell. Beyond that level, the cell in suspension almost always will be forced into the first obvious morphology change, as seen by phase contrast microscopy. (4) Once stoichiometries are established, the total binding capacity of erythrocytes for such compounds, in blood, can be estimated. An intruding organic molecule would encounter about 6 times as many plasma albumin sites as erythrocyte sites, if the plasma albumin sites were free. However, because albumin in vivo usually forms a complex with one to two fatty acids, the erythrocyte itself is rather likely to act as a transport particle for such compounds.

The normal human erythrocyte is a flexible biconcave disk. Various compounds convert it to other morphologies in suspension, observable with phase contrast microscopy. The sequence of appearance is: flexible biconcave disk = crenate shape, or a cup shape, depending on the compound = sphere = massively leaking cell = ghost. The reverse arrows indicate that frequently a distorted morphology can be returned to the normal biconcave disk shape. Most compounds which cause the first morphology change beyond the flexible biconcave disk fall into the classification given by Deuticke (1). For example, alkylsulfonates cause formation of spiky crenates, or echinocytes.1 Organic cations usually induce smooth cup shapes, or stomatocytes. Reversal from the crenate is often promoted by plasma albumin, which is especially effective in binding organic anions. Ponder (4) described and illustrated in some detail a number of the stages in the biconcave disk to sphere conversion, through crenate intermediates.

A rather small fraction of all of the literature on erythrocyte-compound interaction is devoted to the study of intact erythrocyte behavior as a function of numbers of molecules bound. Of the binding data which is available, much is with the ghost. The intact human erythrocyte, from our data, can engage in two kinds of binding behavior that are very much different from one another, particularly at lower levels of binding. One of these is statistical, or normal binding, with negative sloping Scatchard plots throughout. The other is cooperative binding behavior, with strongly positively sloping Scatchard plots in the initial sections of the binding isotherm. Statistical binding usually indicates that ligands bind in a progressive way to strong sites first, the weaker sites later but with all of the sites available throughout. Cooperative binding corresponds to initial resistance to binding, with much increased binding later. Probably there is exposure of more sites as binding proceeds. We show that there is rather close correspondence between what looks like exposure of sites, judged by maxima in Scatchard plots, and profound

1 A trend in erythrocyte morphology description is to use the terminology of Bessis and Lessin (2) and Bessis et al. (3).
morphology changes, judged by phase contrast microscopy of erythrocytes in hanging drop suspension.

Some striking papers by Seeman and colleagues (5, 6) showed that a number of the drugs used in the present work protect the red cell from hemolysis during a period of osmotic shock. Briefly, the results are that if carefully handled, fresh human cells are exposed to ionic strengths of 0.060 to 0.070 at 25° for a few hundred seconds, up to 70% hemolysis occurs. But if the experiments are carried out with predominantly nonpolar compounds, rigorously controlling ionic strength and the interval of shock, with optimum total drug concentrations ranging from 10⁻⁴ m to 10⁻² m depending on the compound, then much less hemolysis occurs. Often, hypotonic hemolysis with drugs can be held to only 5 to 10% lysis, in contrast to 70% hemolysis without drug. We have repeated a number of these experiments with drugs, confirming the results in most cases. There are two additional points: (a) calcium also protects the cell, particularly at physiological calcium levels. Van Kampen et al. (7) observed this some time ago. (b) The optimum drug concentrations that it takes to protect the erythrocyte correspond to binding levels which are adequate to force most of the cells into their first distinctive morphology beyond that of the normal biconcave disk (discocyte). At these levels, the cells are either spiky crenates or cup-shaped. It seems likely, therefore, that the maximally protected erythrocyte is a distorted erythrocyte.

Another aspect of determination of stoichiometries and mechanisms of binding has to do with the possibility that the red cell transports compounds in the blood. Its average binding capacity needs comparison with plasma albumin, taking into account the hematocrit and the total amount of albumin. These comparisons are made below.

**EXPERIMENTAL METHODS**

The erythrocytes, from venipuncture or finger lance, were drawn into 0.9% NaCl including 1 unit of heparin per ml of blood, or into 50 volumes of saline (0.9% NaCl solution), respectively. The blood was washed three times by centrifugation in saline, and in most experiments were used in 1 to 6 hours. No glucose was added. The binding data was not dependent on cell storage time after the last wash, in this time range. Cell concentrations from stirred suspensions were usually obtained by measurement of hemoglobin content, with occasional checks by Levy-Hausser plate counting and Coulter counter. In the hypotonic shock protection experiments, cell concentrations were 5 x 10⁸ cells/ml before mixing. In binding equilibria experiments, final cell concentrations were 5 to 10 x 10⁹ cells/ml depending on the compound used. Cells from different individuals were used, but these were not mixed or saved. The compounds and drugs were characterized physically, usually by spectrophotometry and comparison with the literature. There were two criteria of the care used in handling the cells, preparing the glassware, etc. These were the duplication of a number of Seeman’s (5) hypotonic shock protection results, and retention of nearly 100% biconcave disk morphology in phase contrast microscopy. The samples were viewed in hanging drops at 25 ± 2°. With the hanging drop, minimal cell to glass surface contacts occur during the counting procedure. Such contacts caused marked morphology changes as Ponder (4) pointed out. The situation is not improved by use of siliconized surfaces, as Deuticke (1) noted. The counting of cells to obtain the fraction of conversion to distorted morphology from the normal cells was done by counting of both morphologies in the mixture, a biconcave disk count plus a count of the distorted cells. Fig. 1 shows photographs of intact cells and of samples which have been converted in various degrees to distorted morphologies.

The hypotonic shock experiments were carried out in glass-stoppered Y tubes. The tubes were incubated in a temperature-controlled bath (25°) for 10 min before and after mixing. The general methods paralleled those of Roth and Seeman (6) except that we used 1 unit of heparin in place of their 50 units, per ml of initial blood. The compound of interest was incubated with cells in isotonic salt 15 mm Tris-HCl at pH 7, for 10 min before mixing. In the low salt branch of the Y tubes, the drug concentration was kept equal to that of the isotonic, drug incubated cells before mixing. In the experiments involving variation in calcium ion concentration, with Tris buffer and supporting electrolyte, all of the ionic species were taken into account in order to rigorously maintain total ionic strength at both the upper and lower tunicity levels. These were 0.169 and 0.072, respectively. We define relative hemolysis level 1.0 as the level at which 70% of all available erythrocyte hemoglobin leaked in the time interval 300 s. In experiments involving protection against cell leakage of protein in isotonic solution, protein analyses were carried out by the Hartree-Lowry method, defined in Ref. 8. The binding experiments employed centrifugation of the erythrocytes after incubation of cells with a buffered saline solu-
The ability of numerous compounds to protect the erythrocyte against fairly severe hypotonic shock is illustrated by propranolol in Fig. 2. Propranolol reduces the fraction of cells hemolyzed to 15%, relative to the case in which the same shock is imposed with no drug. The optimum level of total propranolol concentration for protection occurs at about $5 \times 10^{-4} \text{M}$. This concentration decreases hemolysis at an optimum level. This occurs, perhaps fortuitously, in the range between free and total physiological calcium, 1 to 5 mM. As noted under “Experimental Methods,” total ionic strengths must be carefully controlled throughout the range. As CaCl$_2$ concentrations increased, buffer concentration was kept constant. The NaCl concentrations were adjusted accordingly, in achieving the stated ionic strengths at both levels used.

Quantitating the fraction of cells converted to a changed morphology, as a function of amounts of drug or compound bound, allows extrapolation to the level of binding which is required to convert all or nearly all of the cells to the changed morphology. This is shown in Fig. 3 with chlorpromazine, a cup inducing compound. Per cent conversion of normal biconcave disks to cups is plotted versus $v_p$ both the added amount of chlorpromazine per cell, and $v_p$, the amount bound per cell. In Fig. 3, there is shown the short extrapolation to the amount bound per cell at complete conversion. This is $v_L$. Values of $v_L$ are given for other compounds in Table I. Chlorpromazine, propranolol, 5-tert-octyl salicylate, quinine, and dodecylbenzene sulfonate all fall in the range $1 \times 10^7 < v_L \leq 4 \times 10^7$.

Binding data for the compounds were plotted according to Scatchard’s method, $v/C$ versus $v$, as it is sensitive to various kinds of behavior (10). $C_f = \text{concentration of free, nonbound substrate}$. The organic cations which lack flexible aliphatic chains, quinine and methylene blue, yield normal appearing concentrative strong binding, $C_f \sim$ experimental error

| Binding behavior | Compound | Parameters | First morphology beyond normal biconcave disk |
|------------------|----------|------------|-----------------------------------------------|
| Cooperative binding, as in Fig. 3, positive slope | Phenanthrene | $v_p = 4 \pm 1 \times 10^7$<br>$v_p = 20 \pm 4 \times 10^7$ | Crenate<br>Crenate |
| Cooperative binding, as in Fig. 4, with large enough range to reveal the biphasic character | 5-Bromosalicylate | $n = 1.5 \pm 0.5 \times 10^7$<br>$n = 2.8 \pm 0.3 \times 10^7$<br>$n = 2.5 \pm 1 \times 10^7$<br>$n = 3.1 \pm 1 \times 10^7$<br>$n = 4 \pm 2 \times 10^7$<br>$n = 2 \pm 1 \times 10^7$<br>$n = 6 \pm 1 \times 10^7$ | Crenate<br>Bumpy crenate<br>Cup<br>Cup<br>Spiky crenate<br>No marked morphology change<br>Cup<br>Spiky crenate |
| Statistical or normal binding, negatively sloping plot throughout | Salicylate | $K = 4.3 \pm 0.8 \times 10^7 \text{m}^{-1}$ | Crenate<br>Bumpy crenate<br>Cup<br>Cup<br>Spiky crenate<br>No marked morphology change<br>Cup<br>Spiky crenate |
| Quantitative strong binding, $C_f \sim$ experimental error | Chlorpromazine | $K = 3 \times 10^7 \text{m}^{-1}$<br>$v_L = 4 \pm 1 \times 10^7$<br>$v_L = 1.5 \pm 1 \times 10^7$ | Crenate<br>Bumpy crenate<br>Cup<br>Cup<br>Spiky crenate<br>No marked morphology change<br>Cup<br>Spiky crenate |
FIG. 3. Phase contrast microscopy counting of morphology, dependent on total and bound levels of chlorpromazine (CPZ). Isotonic solution, pH 7, 25°. Extrapolation to obtain ρL, the amount bound at complete morphology conversion.

FIG. 4. Scatchard plot for phenanthrene binding. Cooperative binding through the range of binding up to the limit of phenanthrene solubility. Determination of ρP.

Scatchard plots. That is, the slopes are negative throughout even if the plot has some biphasic character. From this, it is generally assumed that binding is normal, or statistical in nature, with the strongest sites binding first. The plots give an average K value for association to the cell, and the value n, which is the abscissa intercept, corresponds to a number of sites. Such values are gathered in Table I, for methylene blue and quinine.

Powerfully binding detergents such as dodecylbenzene sulfonate (DBS−) leave so little compound free in solution that despite the improvements of Gould's method in detergent analysis (9), unavoidable experimental error magnitudes approach the total values sought. The method was found to be quantitative in the range recommended by Gould. However, dodecylbenzene sulfonate binding leaves amounts free in solution, Cf, below that range. Hence, the denominator in ϱ/Cf fluctuates too much to yield reliable Scatchard plots.

Nevertheless, the measurements show that nearly all added dodecylbenzene sulfonate is bound. We cannot precisely measure Cf, but we nearly quantitatively obtain ϱ. It is between 96 and 100% of the dodecylbenzene sulfonate added in the 10−3 M range of total dodecylbenzene sulfonate. Hence our work supports Ponder's calculations (4) of erythrocyte surface area coverage by similar detergents, using them to force morphology changes. In his work (some time before Gould's method became available), the assumption was made that all detergent added was bound. In short, Ponder's (4) assumption is confirmed as being correct, for practical purposes. A second consequence is that, since nearly all dodecylbenzene sulfonate is bound up to the level at which the morphology change to a crenate occurs, it is a simple matter to obtain ρL for dodecylbenzene sulfonate. It is given in Table 1.

For most of the compounds other than dodecylbenzene sulfonate, binding equilibrium data and Scatchard plots were more easily obtained. Some of the compounds produce negative sloping Scatchard plots, as expected for normal progressive binding to stronger sites first and weaker sites later. Other Scatchard plots, however, are of a markedly different kind. They have an initial, quite positive sloping character, as shown by Figs. 4 and 5. This behavior is quite unaccounted for by a simple equilibrium: \( S + \text{cell} \leftrightarrow \text{bound } S \), with \( S = \text{free compound} \). This remains the case, even with multiple sites, if the model is taken as one with normal statistical binding with the strong sites being exposed at the start, where \( \rho = 0 \). In the case of phenanthrene, Fig. 4, limited solubility of the compound prevents observations at \( \rho \) values larger than those shown. The plot would take on a negative character at hypothetical large values of \( \rho \). However, short of that, there occurs a binding level where the plot plateaus, called here \( \rho_P \). This level marks the end of the first stage of binding of the phenanthrene, which is cooperative in nature. That is, there is a strongly positively sloping section of Scatchard plot during the first stage of binding to the cell, much as occurs with some binding processes at the molecular level.

Chlorpromazine and propranolol give Scatchard plots with quite biphasic character: a cooperative section with a positive slope, followed by normal binding (apparently normal binding), with a negative sloping section in the plot. This is shown in Fig. 5. The point on the abscissa at which the mode of binding changes markedly is \( \rho_w \). The \( \rho_w \) point, \( \rho_w \), is about \( 2 + 1 \times 10^4 \)
molecule/cell, and this is the case for a number of compounds, despite the diversity in the over-all or total levels at which they exert their effects on the cell. Values for \( n_s \) are listed in Table I.

It is often impractical to drive the compound levels high enough to extrapolate very well to the abscissa from the negative sloping section of the plot. For \( n_s \) already coincides with the first discernible morphology change; at much higher binding levels, drug-induced hemolysis frequently occurs. Fig. 5 may give the appearance of extrapolating to the origin in the early positive sloping section. Actually there should be an intercept on the ordinate as \( v \to 0 \), at least for reasonably simple models. This is discussed below, and is more clear in the phenanthrene case (Fig. 4).

The washed red cell continually leaks, or sloughs off from its surface, a small but measurable amount of protein, perhaps "peripheral protein" in the Singer and Nicolson (11) description. We found that about \( 4 \times 10^{-9} \mu g \) cell\(^{-1} \) min\(^{-1} \) of protein leaked, when no protecting agent was added to washed isotonic cells. However, with Na\(^+\) dodecylbenzene sulfonate added at levels just below those needed to promote the production of mostly crenated cells, the protein leakage rate drops to about one-half that with no detergent. This occurs in the region of \( 2 \pm 1 \times 10^7 \) detergent added per erythrocyte, which virtually amounts to a number bound per erythrocyte, as noted above. At appreciably larger dodecylbenzene sulfonate levels, in the region \( 5 \times 10^7 \) anions per cell, extensive protein leakage and lysis occurs. Thus it appears that the optimum level for protection of erythrocytes against hypotonic shock is also optimum for protecting against isotonic protein loss, for the case of Na\(^+\) dodecylbenzene sulfonate.

**DISCUSSION**

It appears from our work so far that there is a marked commonality of numbers of sites around which are hinged various kinds of behavior. This can be expressed by summarizing a considerable amount of our own data by the range and relationship:

\[
8 \times 10^7 > n \sim n_r > n_2 \sim n_s > 1 \times 10^7 \quad (1)
\]

Probably there is no fixed number of sites. The apparent number may depend on the compound and the changes set in motion upon binding. The binding level \( 2 \pm 1 \times 10^7 \) is one at which the cells have generally taken on a maximum amount of charged organic compound, either cationic or anionic, before they will almost certainly be converted to a new morphology, cup or crenate. At \( v \sim 2 \pm 1 \times 10^7 \), the cells also tend to be maximally protected against hypotonic strain. If they engage in cooperative binding, as they often do, it is also at \( v \sim 2 \pm 1 \times 10^7 \) at which filling of the later sites starts. This perhaps occurs as a result of exposure of the later sites, or perhaps as a result of red cell acquiescence to admitting the compounds past the membrane to go on inside. It is known that hemoglobin binds a number of the compounds studied here (12), and binds aliphatic alkanes (13).

Estimates of the number of sites also can be found from the literature, if one includes what are often conditions rather different from our own. These are summarized in Table II. Admittedly, comparison of intact erythrocytes with the ghost, and at somewhat differing temperatures, makes for only rough comparisons. However on the average, stoichiometries which can be gleaned from other work fall rather well into Equation 1, above.

From our own work, and that of some of the literature, it is fairly likely that long flexible hydrocarbon compounds, especially strong anions, are stopped at the membrane. The prototype is alkylsulfonate detergent. With more rigid hydrocarbon compounds, neutral or positively charged, there is a chance of passive transport, possibly accompanied by cooperative binding as in Figs. 4 and 5. Methylene blue is an example. It is apparent, however, that there is lacking quantitative data for many higher molecular weight compounds. If these data were available, it would enable one to make reliable predictions concerning whether a compound which binds is confined to the surface, or gets inside.

Two precautions need use in viewing the data. First, although cells were not pooled from various donors in each set of experiments, the cells are still heterogeneous with respect to age, in each donor's cells. Therefore, it is possible that the average behavior seen may be skewed toward the behavior of younger or toward older cells. Second, processes which accompany binding of drugs and morphology changes, notably K\(^+\) leakage, may be involved. For instance, Lichtman et al. (14), Manninen (15), and Roth and Seeman (16) studied K\(^+\) outflow caused by various drugs. At this point, it is uncertain whether it is a prime contributor to drug-induced morphology changes, or whether it is a consequence, or effect, of drug-membrane interaction such as drug inhibition of the Na\(^+\)-K\(^+\) pumps. Nevertheless, K\(^+\) exodus accompanies the process, as do water transport and nonisovolumetric changes. In short, there may be several reactions set in motion which remain to be quantitated, and put in relation to one another, that accompany binding and morphology changes.

The modes of binding of erythrocytes have analogs at the molecular level, with proteins. Statistical binding is characterized by negatively sloping Scatchard plots, the strongest sites binding first with the steepest slopes. There are increasing numbers of examples of enzymes and proteins which utilize cooperative

| Table II |
| Number of sites on human red cell for compound binding; literature values |

| Systems | Conditions | Number of sites | Reference |
|---------|------------|----------------|----------|
| C\(_4\) to C\(_8\) normal alcohols, erythrocyte | 22° | 1 to 8 \times 10^7 depending on alcohol | Seeman et al. (18) |
| | 22° | 5 \times 10^7 | Kwant and Seeman (19) |
| Chlorpromazine, erythrocyte ghost | | | Spector (22) |
| Laurate fatty acid anion, intact cells | 37° partitioning with plasma albumin | \( n_1 = 0.01 \times 10^7 \) | |
| | | \( K_1 = 1.8 \times 10^6 \) | |
| | | \( n_2 = 0.78 \times 10^7 \) | |
| | | \( K_2 = 1.3 \times 10^6 \) | |
| | | \( n_1 + n_2 \sim 0.8 \times 10^7 \) | |
| Na\(^+\) tetradeceylsulfate | Discernible crenation \to extensive crenation \to crenate sphere | 0.5 to 3 \times 10^7 | Ponder (4) |
binding, which with Scatchard plotting give positive sloping plots; the apparently weaker sites binding first. The simplest model with which the matter may be viewed is one in which a protein or cell, $P$, binds substrate $S$, using only two sites with $K_1$ and two equilibria in sequence: $P + S \rightleftharpoons PS_1; PS_1 + S \rightleftharpoons PS_2$. If $K_2 > K_1$, it can be shown using an analysis similar to that of Cassman and King (17), that $\lim_{x \to \infty} \frac{C_p}{C_i} = \frac{1}{K_2 - K_1}$, in such a sequence. The quantity $[d(C_p/C_i)/dv]$ is the Scatchard plot slope. The initial slope will be positive if $K_2 > 0.5 K_1$ and negative if $K_2 < 0.5 K_1$. At the other extreme where all the sites are filled, $\lim S \to \infty$, the slope is $(\sim)$ as one might expect. Hence even with only two binding sites, biphase Scatchard plots might occur. A model with only two kinds of sites may be oversimplified, particularly with cells. However, with multiple classes of sites, if "buried" or cryptic but stronger sites exist or arise as a result of membrane changes, a positive sloping Scatchard plot over part of the binding range is expected. This may have already been seen with the erythrocyte ghost. Seeman et al. (18) reported a $K$ from Scatchard plotting which was often apparently negative for the octanol-ghost interaction. Upon replotting Kwant's and Seeman's data (19) for the chlorpromazine-ghost system, in a Scatchard plot, a positive sloping section apparently occurs. Perhaps Scatchard plotting is sometimes avoided because of the occurrence of positive slopes and apparently negative association constants. But such behavior simply reveals interesting aspects of the protein's or cell's behavior. The particle is more complex than a static particle with simple statistical binding. Initially, the particle binds relatively weakly. In neither the simplified sequence above, nor from our data, insofar as it extends, is there necessarily passage of a Scatchard plot through the origin. In the two-site sequential model above, $\lim_{x \to \infty} \frac{C_p}{C_i} = K_1$, which is the ordinate intercept. The first substrates which bind, however, perturb the intact cell. At some point, the binding mechanism possibly changes, with increasing $v$ eventually leading to apparently normal or statistical binding and negative sloping Scatchard plot behavior.

As noted above, often $v_L \sim v_L$, especially for certain drugs. Thus the events leading to a morphology change probably are connected with those leading to cooperative binding. Both phenomena may involve what in effect is an exposure of later, fairly strong ($K \sim 10^4 M^{-2}$) binding sites after initial loading of the first sites.

Concerning the fraction of surface coverage of the erythrocyte, we may take its normal area as $1.5 \times 10^{10}$ (A)² (20). If $v \sim 2 \times 10^6$, there is available to each bound molecule about 700 to 800 (A)² at such a binding level. The areas of a number of organic compounds, in the range molecular weight = 300 to 600, cover 100 to 200 (A)² if they lie flat. It is seen that if the cell accumulates compounds which approach the level $v \sim 2 \times 10^7$, then an appreciable fraction, 10 to 20%, perhaps, of the cell's surface may become covered. If $v > 2 \times 10^7$, there could be approached nearly monolayer coverage, if the molecules reclined or lie flat. At this level, it is perhaps not surprising that a major morphology change ensues. The membrane is likely to have been perturbed considerably when such large fractions of its surface have been made to accommodate foreign molecules.

It is useful to compare the binding capacity of the erythrocyte for compounds in general with that of blood proteins, especially serum albumin. Such comparisons depend on the numbers of sites assigned to the cell and to the protein molecules, respectively. From the many studies of plasma albumin, there appear to be about two strong sites per molecule. Using the foregoing results, there are potentially available $2 \times 10^7$ sites per normal red cell. Both kinds of particles have additional sites for most compounds. But if one considers the plasma albumin concentration, and the hematocrit of blood, there would be in 1 ml of blood, about $1 \times 10^7$ erythrocyte sites and about $6 \times 10^7$ albumin protein sites. It might appear that an intruding organic molecule would tend to bind mostly to the protein. For the erythrocyte often tends to bind such compounds in a cooperative manner, initially resisting binding, when $v \sim 0$, and then acquiring when $v \to v_L$ or $v_v$. However, the matter is more complicated (and interesting) than that. It now appears that plasma albumin binds certain compounds in a cooperative manner² (21). Further, in vivo, blood plasma albumin is normally already partly loaded with fairly tightly bound compounds, especially fatty acids (22). Goodman (23) considers that $v \sim 1$ for albumin-fatty acid in plasma. In sum, a molecule appearing in the blood is probably likely to become partly bound to red cells. The situation may vary considerably depending on competing ligands, the structure of the compound, and the extent of loading of the plasma albumin, as Spector (22) indicates.

Acknowledgment—We thank Dr. John Gander for his aid. Drugs were donated by the Ayerst Co. and the Smith, Kline and French Co.

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² Replotted in extenso from data in the literature and unpublished data here on plasma albumin-organic-ion interaction, exhibit cooperative behavior in numerous examples.
Stoichiometry of compounds bound to human erythrocytes in relation to morphology.
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