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SURFACE AFFINITY CHROMATOGRAPHIC SEPARATION OF BLOOD CELLS

IV*. RELATIONSHIP BETWEEN SURFACE HYDROPHOBICITY OF HUMAN PERIPHERAL BLOOD CELLS AND THEIR RETENTION BEHAVIOUR ON POLYETHYLENE GLYCOL 20M-BONDED SEPHAROSE COLUMNS

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SUMMARY

The partition behaviour of human peripheral blood cells was studied in dextran T40–polyethylene glycol (PEG) 6000 two-phase systems with and without PEG 6000 palmitate as a hydrophobic ligand at pH 7.5 and at the isoelectric points for the four kinds of cells. $\Delta \log K$, where K is the partition coefficient, is taken as a measure of the hydrophobic interactions between the cell surface and the palmitoyl residue. Its value is dependent on the concentration of palmitate in the partition systems and on the kinds of cells, increasing in the order platelets, granulocytes, lymphocytes and erythrocytes. A linear relationship was found between the retention volumes of these cells, except that of erythrocytes, on a PEG 20M–Sepharose column and their $\Delta \log K$ values determined in the presence or absence of sodium chloride both at the isoelectric points and at pH 7.5.

INTRODUCTION

The chromatographic separation of human peripheral blood cells has been achieved by using an oxirane-coupled polyethylene glycol (PEG) 20M–Sepharose 6B (PEG 20M–Sepharose) column and a phosphate buffer solution (pH 7.5) containing dextran T40 or T500 as the mobile phase[1,2]. The blood cells were eluted from the column in the order erythrocytes, platelets, granulocytes and lymphocytes. An increase in sodium chloride concentration with a concomitant decrease in sodium phosphate buffer concentration in the mobile phase while maintaining the isotonicity resulted in an increase in the retention volumes of erythrocytes, granulocytes and lymphocytes[2]. It is thought that the retention behaviour of the cells must depend on

* For Part III, see ref. 6.

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properties other than the membrane surface charge of the cells, because in the presence of the neutral salt the mobile phase and the stationary PEG phase provide little electrostatic potential difference with which the membrane charges could interact. In addition, it is known that, in an aqueous polymeric two-phase (APTP) system, sodium chloride is partitioned almost equally between dextran and PEG phases[3,4]; thus, this system has virtually no electrical potential between the phases[5].

It should be noted that the retention behaviour of blood cells on the column described above does not depend on the size, charge or adhesiveness of the cells. In Part III it was shown[6] that the retention volumes of lymphocytes, granulocytes and platelets showed a marked dependence on the molecular weight of the bonded PEG in the range of 400–20,000, increasing with increasing molecular weight. The same tendency was observed when the pH of the mobile phase used in this chromatographic system was adjusted to one of the isoelectric points determined for the four kinds of blood cells by using the cross-partition method. It was suggested that the hydrophobic interactions between the hydrophobic moieties on the cell surface membrane and the bonded PEG phase were probably the main factor affecting the retention on these columns of lymphocytes and granulocytes at their isoelectric points. On the other hand, for erythrocytes and platelets, both electrostatic and hydrophobic interactions seemed to be important, at least at pH 7.5.

It has been reported that hydrophobic affinity partition in an APTP system can be applied to the measurement of the hydrophobicity of water-soluble proteins[7]. This involves partition of the proteins in a dextran–PEG system where some of the PEG is esterified with fatty acids that provide a hydrophobic ligand. Proteins that interact with the hydrocarbon residues will favour partition in the PEG-rich upper phase. The difference, $\Delta \log K$, between the logarithm of the partition coefficients of the protein in systems with and without fatty acid ester is taken as a measure of the hydrophobicity. Eriksson *et al.*[8] distinguished erythrocytes from various species and classified them in two groups based on their affinity for the PEG ester; it was suggested that the ability of the PEG ester to bind to erythrocytes may be species-dependent. In practice, however, they did not determine the $\Delta \log K$ values.

In this study, we have applied this hydrophobic affinity partition method to human peripheral blood cells in order to specify their surface hydrophobicities.

EXPERIMENTAL

Materials

Dextran T40 (weight-average molecular weight, M_w = 40,000) was obtained from Pharmacia (Uppsala, Sweden). Polyethylene glycol (number-average molecular weight, M_n = 6000–7500) was purchased as PEG 6000 (extra pure grade) from Wako (Osaka, Japan). Other reagents were of analytical reagent grade.

Polyethylene glycol palmitate (PEG palmitate) was synthesized from PEG 6000 and palmitoyl chloride in the presence of triethylamine in toluene solution[9]; *ca.* 60% of the total hydroxyl groups in the PEG were substituted.

Instruments

A Coulter Counter Model D (Coulter Electronics, Harpenden, U.K.) was used for counting the number of blood cells.

Collection and isolation of blood cells

Human blood was drawn from normal male adult donors by venous puncture and heparin was added, 0.05 ml of a 1000 U/ml solution per 10 ml of blood. Siliconized glassware was used in all procedures.

Erythrocytes. Blood was centrifuged at 500 *g* for 10 min and the supernatant and the buffy coat layer were removed. The cells were washed three times with 0.9% (w/v) saline.

Platelets. A centrifugation isolation technique based on that of Leeksma and Cohen[10] was employed. Platelet-rich plasma (PRP), the supernatant of heparinized whole blood centrifuged at 65 *g* for 20 min, was centrifuged at 250 *g* for 20 min. The partly sedimented platelets were resuspended, the PRP was centrifuged at 250 *g* for 20 min and the sedimented platelets were then washed thrice with 0.9% (w/v) saline to remove other contaminating cells.

Granulocytes. The sodium metrizoate–dextran T500 sedimentation technique[11] was used. The granulocyte preparation contained a variable amount of contaminating erythrocytes.

Lymphocytes. The sodium metrizoate–Ficoll sedimentation technique[12] was used. The erythrocyte contamination of the lymphocyte preparation was usually between 1 and 5% of the total number of cells.

The above isolation procedures have been described in detail elsewhere[1,2].

Retention volumes of blood cells

Table I shows the retention volumes of the four kinds of blood cells on a PEG 20M–Sepharose 6B column (25 × 0.9 cm I.D.) at pH 7.5 and at the isoelectric points which were determined[6] by using a cross-partition method. The pH values at the cross-point, $pH_{c.p.}$, were found to be 5.5 for erythrocytes, 6.8 for platelets and granulocytes and 5.2 for lymphocytes, respectively. The retention volumes in Table I are quoted from our previous paper[6]; they were obtained by using sodium phosphate buffer solution (0.09 *M*) containing 2% (w/w) dextran T40 as the mobile phase, and were averages from several measurements.

TABLE I

RETENTION VOLUMES OF HUMAN PERIPHERAL ERYTHROCYTES (e), PLATELETS (p), GRANULOCYTES (g) AND LYMPHOCYTES (l) ON A PEG 20M–SEPHAROSE 6B COLUMN AT pH 7.5 AND AT THEIR ISOELECTRIC POINTS[6]

Mobile phase: 2% (w/w) dextran T40 buffered with 0.09 *M* sodium phosphate. Isoelectric points, $pH_{c.p.}$: pH 5.5, erythrocytes; 6.8, platelets and granulocytes; 5.2, lymphocytes. Column: 25 × 0.9 cm I.D., oxirane-coupled PEG 20M–Sepharose 6B treated with 0.1 *M* perchloric acid to hydrolyze residual epoxy groups after the coupling.

pH of mobile phase	*Retention volumes (ml)*			
	e	*p*	*g*	*l*
7.5	5.8	7.2	13.2	15.9
Isoelectric points	5.6	6.5	11.4	16.0

Hydrophobic affinity partition of blood cells

Two-phase systems and partition. Dextran T40 and PEG 6000 were used according to Eriksson *et al.*[8]. However, the concentration of PEG 6000 in the system was modified from 4 to 8% (w/w) to facilitate the phase separation.

The "sodium chloride system" contained 8% (w/w) of dextran T40, 8% (w/w) of PEG 6000, total including PEG palmitate, 0.15 *M* sodium chloride and 0.01 *M* sodium phosphate buffer, pH 7.5 or $pH_{c.p.}$ 5.2, 5.5 and 6.8 for the blood cells. The "without sodium chloride system" comprised the same concentrations of dextran T40 and PEG 6000 as in the sodium chloride system and isotonic 0.13 *M* sodium phosphate buffer, pH 7.5, 5.2, 5.5 and 6.8. All the solutions were filtered through a Millipore filter (pore size 0.47 μm) prior to use to remove particles which might be mistaken for the cells with a Coulter Counter.

The phase systems of total weight 8.0 g were prepared by mixing 3.2 g of a 20% (w/w) dextran T40 solution with 3.2 g of a 20% (w/w) PEG 6000 solution [including 0–25% (w/w) of PEG palmitate in the total PEG 6000; 0–2.0% (w/w) palmitate in the final phase systems] and then adding sodium phosphate buffer with or without 0.07 g of sodium chloride. A 0.1-ml volume of the blood cell suspension in the buffer solution was included in the 8.0 g of the phase systems. The phases were gently mixed by 30 inversions and were allowed to separate for 30 min at room temperature, 20–24°C. After phase separation, 1 ml of both the upper and the lower phases were carefully removed with a pipette. An aliquot of each phase was diluted with 4 ml of Isoton (aqueous electrolyte diluent for blood cell counting; Coulter Diagnostics, Hialeah, FL, U.S.A.) and the number of blood cells was counted with a Coulter Counter. For erythrocytes, granulocytes and lymphocytes, the cell number was measured with an orifice tube of 100-μm aperture, and for platelets the tube had an 70-μm aperture. Corrections of the number of platelets determined by using 1 ml of the sample were made with a calibration table.

Partition coefficient of cells. Partition of the blood cells was characterized by the partition coefficient, *K*, calculated as the ratio of the cell concentration (cell number per ml) in the upper phase to that in the lower phase. The number of the cells attached to the interface was calculated as the difference between the total number added and the number in the upper or the lower phase. Some of the partition data are shown as the percentage of the number in the upper phase or the interface with respect to the total added.

The partition experiments were repeated more than five times for each sample, and the cell concentration determined in the phases deviated from the mean value by not more than 3% for all the cells studied.

RESULTS

Hydrophobic affinity partition of blood cells

Fig. 1 shows the dependence of the blood cell concentration on the partition in the two-phase systems containing 8% (w/w) of dextran T40, 8% (w/w) of PEG 6000 with or without 10% (w/w) of PEG palmitate in the total PEG, 0.15 *M* sodium chloride and 0.01 *M* sodium phosphate buffer, pH 7.5. In the absence of PEG palmitate, the four different blood cells, although of varying extents, are distibuted predominantly in the dextran-rich lower phase or at the interface. The partition data

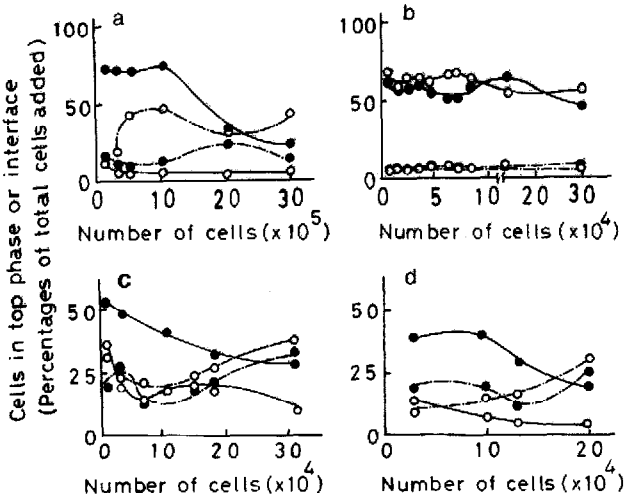

Fig. 1. Dependence of partition on the concentration of human erythrocytes (a), platelets (b), granulocytes (c) and lymphocytes (d) at pH 7.5. Partition was expressed as a percentage of the cells in the upper phase (——) or the interface (- · - ·) of the total cells added. Systems: ○, 8% (w/w) dextran T40, 8% (w/w) PEG 6000, 0.15 *M* sodium chloride and 0.01 *M* sodium phosphate buffer, pH 7.5; ●, 10% (w/w) PEG 6000 was replaced by PEG monopalmitate (0.8% of the final concentration in the system).

for the lower phase are not shown. When PEG palmitate, which itself favours the PEG-rich upper phase, is incorporated into the phase system, those cells that interact with the palmitate are partition in the upper phase. As shown in Fig. 1, hydrophobic affinity partition of the cells thus occurs. On increasing the number of cells added their relative concentration in the upper phase decreases, whereas that at the interface increases or remains nearly constant. The decrease in partition of the cells, except for platelets, in the upper phase may be due to saturation of the binding of the cells to PEG palmitate. In order to obtain an observable difference in the partition of blood cells in the phase systems with and without PEG palmitate, the cell number should be chosen so as to produce both a higher distribution in the upper phase in the presence of PEG palmitate and a lower distribution at the interface in the absence and the presence of the palmitate. The cell number adopted in the following partition experiments was about $2.7 \cdot 10^5$ for erythrocytes, $7 \cdot 10^4$ for granulocytes and $1 \cdot 10^5$ for lymphocytes per total 8 g of the partition systems, respectively. The distribution of platelets, in contrast to the other cells, was little affected by their concentration in the systems because the cell number was reduced to prevent their aggregation. Therefore, platelet numbers ranging from $1 \cdot 10^3$ to $6 \cdot 10^3$ were used.

Fig. 2 shows increasing distribution of the blood cells in the upper phase of the partition system at pH 7.5 and at the isoelectric point, $pH_{c.p.}$, of the cells with increasing concentration of PEG palmitate in the total PEG. The effect of the surface negative charge of the blood cells on the partition may be quenched when the pH of the dextran–PEG system is adjusted to one of the $pH_{c.p.}$ values as determined by a cross-partition method[6]. In Fig. 2, K_p and K_0 are the partition coefficients of the blood cells in systems with and without PEG palmitate, respectively. The four kinds of blood cells show distinctly different partition behaviours in a given two-phase system. The higher K_p values of erythrocytes at pH 7.5 in the PEG palmitate fraction

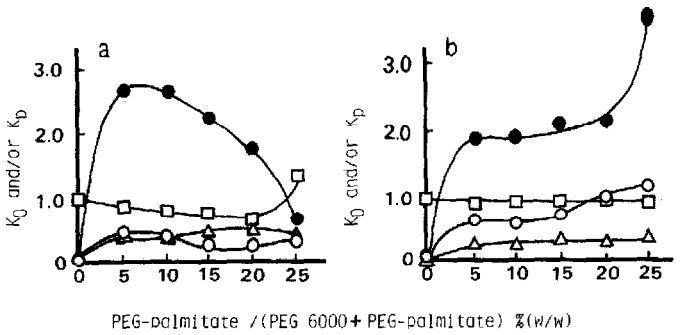

Fig. 2. Changes in partition coefficients of human blood cells as a function of the PEG palmitate fraction of total PEG at pH 7.5 (a) and at the isoelectric points of the cells (b). Blood cells: erythrocytes (●); lymphocytes (○); granulocyates (△) and platelets (□). The partition systems contained 0–25% (w/w) of PEG palmitate in total PEG (0–2.0% of the final concentration in the systems); other details as in Fig. 1. pH values of system[6]: 5.2 for lymphocytes; 5.5 for erythrocytes; 6.8 for platelets and granulocytes.

5–10% (w/w) total PEG are remarkable compared with those of the other cells, but decreased with further increase of the palmitate fraction. The K_p values of lymphocytes and of platelets also increased significantly when the pH was lowered from 7.5 to 6.8 or 5.2, whereas that of erythrocytes decreased in both the 5% and 10% (w/w) palmitate fractions at pH 5.5.

Fig. 3 shows the changes in the $\Delta\log K$ values of the blood cells as a function of the PEG palmitate fraction (%, w/w) of the total PEG 6000 [0–2.0% (w/w) palmitate in the final partition systems]. The effect of PEG palmitate on the hydrophobic affinity partition can be expressed in terms of $\Delta\log K$, defined as $\Delta\log K = \log K_p - \log K_0$[7]. The $\Delta\log K$ values in Fig. 3a were obtained at pH 7.5, and those in Fig. 3b were obtained at the respective $pH_{c.p.}$ of the blood cells. The $\Delta\log K$ curves for granulocytes and lymphocytes intersected at pH 7.5, whereas the difference in the $\Delta\log K$ values at the $pH_{c.p.}$ was distinct. Of the blood cells studied, erythrocytes show the highest $\Delta\log K$ and platelets the lowest value at all the different PEG palmitate contents examined. The change in $\Delta\log K$ of the cells at their $pH_{c.p.}$ as a function of the palmitate content was not the same as that obtained at pH 7.5, in particular, at higher palmitate fractions. In other words, the $\Delta\log K$ values, the increments in the partition coefficients caused by the interactions between the cells and the hydrophobic ligand, were not constant with changing pH of the partition systems. On the basis of the results shown in Fig. 3, we used the $\Delta\log K$ values obtained at 5, 10 and 15%

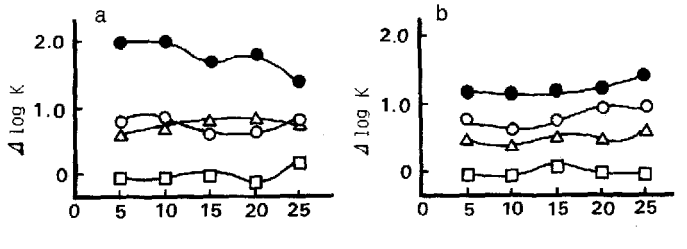

Fig. 3. Changes in $\Delta\log K$ of human blood cells as a function of the PEG palmitate fraction in total PEG at pH 7.5 (a) and at the isoelectric points of the cells (b). Symbols, compositions and pH values as in Fig. 2.

TABLE II

PARTITION COEFFICIENTS, K_0 AND K_p, AND ΔLOG K VALUES OF HUMAN PERIPHERAL BLOOD CELLS IN DEXTRAN–PEG PARTITION SYSTEMS AT pH 7.5 AND AT THEIR ISOELECTRIC POINTS

The number of cells partitioned was $2.7 \cdot 10^5$ erythrocytes, $(1.2\text{–}6.2) \times 10^3$ platelets, $7 \cdot 10^4$ granulocytes, and $1 \cdot 10^5$ lymphocytes, respectively. The common constituents of the partition systems were 8% (w/w) dextran T40, 8% (w/w) PEG 6000 with and without 10% (w/w) PEG palmitate. The sodium chloride system contained 0.15 *M* salt and was buffered with 0.01 *M* sodium phosphate. The system without sodium chloride was buffered with isotonic 0.13 *M* sodium phosphate.

	Erythrocytes		*Platelets*		*Granulocytes*		*Lymphocytes*	
pH	*7.5*	*5.5*	*7.5*	*6.8*	*7.5*	*6.8*	*7.5*	*5.2*
NaCl system								
K_0	0.03	0.13	0.97	0.99	0.08	0.09	0.06	0.07
K_p	2.66	1.91	0.80	0.90	0.40	0.20	0.43	0.30
K_p/K_0	96.80	14.91	0.82	0.91	5.21	2.31	7.20	4.62
Δlog K	1.99	1.17	−0.09	−0.04	0.72	0.36	0.86	0.67
Without NaCl								
K_0	0.19	0.16	1.05	1.38	0.23	0.12	0.14	0.11
K_p	13.51	0.85	1.02	0.96	0.45	0.24	0.47	0.86
K_p/K_0	69.98	5.43	0.97	0.70	1.95	2.01	3.39	7.85
Δlog k	1.85	0.74	−0.02	−0.12	0.30	0.30	0.53	0.90

(w/w) PEG palmitate in the total PEG for comparing the hydrophobicities of the blood cell surfaces.

Table II shows the K_0, K_p, K_p/K_0 and Δlog K values for the four human blood cells in the "sodium chloride system" with and without 10% (w/w) PEG palmitate (0.8% in final the partition system). The partition characteristics obtained in the "without sodium chloride system" will be discussed in a later section.

Relationship between Δlog K and retention volumes of blood cells

The Δlog K values of platelets, granulocytes and lymphocytes determined in the sodium chloride system containing 5, 10 and 15% (w/w) PEG palmitate at pH 7.5 or at the respective $pH_{c.p.}$ values are plotted in Fig. 4 against the retention volumes of these blood cells. The retention volumes were obtained on a PEG 20M–Sepharose 6B column with a phosphate-buffered 2% (w/w) solution of dextran T40, pH 7.5 or $pH_{c.p.}$[6]; the values are summarized in Table I (see Experimental). Fig. 4a shows that an approximately linear relationship was found between the retention volumes and the respective Δlog K values determined in the partition systems containing 5 or 10% (w/w) PEG palmitate. There is no such relation, however, for the system containing 15% (w/w) palmitate. The Δlog K values determined at the respective $pH_{c.p.}$ values when plotted against the corresponding retention volumes yield excellent linear relationship for all three fractions of PEG palmitate (Fig. 4b). Erythrocytes should be excluded from the plots because of their much higher Δlog K values under all partition conditions examined (see Table II). As mentioned above, the dextran–PEG systems used in the partition experiments contained 0.15 *M* sodium chloride to lower

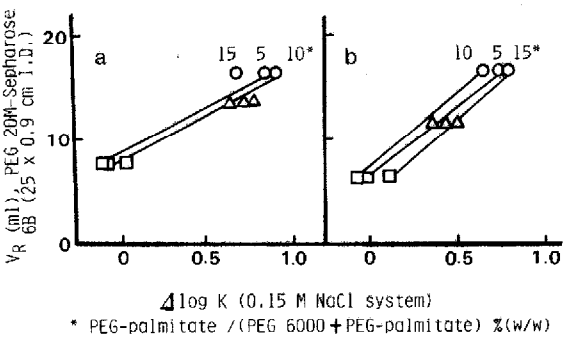

Fig. 4. Relationship between $\Delta\log K$ and the retention volumes of human blood cells at pH 7.5 (a) and at the isoelectric points of the cells (b). Blood cells: platelets (□); granulocytes (△) and lymphocytes (○). The partition systems contained 5, 10 and 15% (w/w) PEG palmitate, PEG palmitate/(PEG + PEG palmitate) %; other details as in Table II. The mobile phase for the chromatography was as in Table I, pH values as in Table I and II.

the partition coefficient, K_0, in the phase system in which PEG palmitate is absent.

Further experiments were made to investigate the causes of the higher $\Delta\log K$ values of erythrocytes, using a phase system in which sodium chloride was absent, because phosphate-buffered dextran solutions containing no sodium chloride are commonly used as the mobile phases in combination with bonded PEG stationary phases[1,2,6]. Table II shows that the partition in this system results in a marked increase in the K_0 values of the blood cells, with the exception of platelets, at any pH value. An increase in the K_p values was also observed for erythrocytes at pH 7.5 and for lymphocytes at the $pH_{c.p.}$; a moderate decrease was observed for the K_p value of erythrocytes at the $pH_{c.p.}$. Even in this system, erythrocytes show a much higher value of $\Delta\log K$ than that of the other cells both at pH 7.5 and at pH 5.5 because of the higher K_p values. Fig. 5 shows the practically linear relationship between the retention volumes and the $\Delta\log K$ values for platelets, granulocytes and lymphocytes in the absence of sodium chloride both at pH 7.5 and at the $pH_{c.p.}$. Thus, the retention of these three kinds of blood cells on the PEG 20M–Sepharose column depends also on their cell surface hydrophobicities. Erythrocytes should be treated as exceptions because of their higher $\Delta\log K$ values.

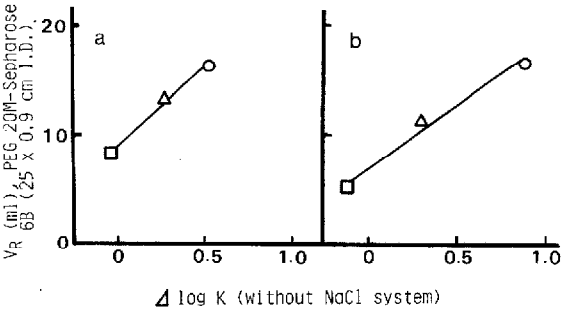

Fig. 5. Relationship between $\Delta\log K$ in the absence of sodium chloride and the retention volumes of human blood cells at pH 7.5 (a) and at the isoelectric points of the cells (b). Symbols of the cells as in Fig. 4. The partition system contained 10% (w/w) PEG palmitate; other details and pH values as in Table II. Mobile phase was as in Table I.

DISCUSSION

It is well known that blood cell surfaces determine important aspects of many physiological and biophysical phenomena and that these phenomena take place principally on the basis of the non-covalent interactions at the cell surfaces. These properties of the blood cell surfaces are referred to as the cell surface hydrophobicity, but this term is not well defined and poorly describes the forces. For soluble proteins, Shanbhag and Axelsson[7] have shown that the partition of serum albumin and also a number of other proteins between the two-phase dextran–PEG system can be influenced significantly by the introduction of straight-chain aliphatic hydrocarbons covalently bound to PEG; the affinity of the proteins for the PEG-rich upper phase increases several-fold. The difference in the partition of albumin in phase systems with and without PEG-bound fatty acid groups provides a measure of the interaction of fatty acids with the protein[7]; the maximum effect is obtained with aliphatic chains containing sixteen carbon atoms[13]. The difference, $\Delta \log K$, between the logarithms of the partition coefficients in systems with and without fatty acid ester is taken as a measure of the hydrophobicity[7].

This hydrophobic affinity partition method has been applied to insoluble, suspended materials such as cell particles, subcellular organelles and cell membranes. It has been pointed out that the partition of chloroplasts[14] and erythrocytes from various species[8] is strongly influenced by hydrophobic ligand groups bound to the PEG in dextran–PEG systems. PEG monopalmitate is most effective in increasing the affinity of different species of erythrocytes to the upper phase, followed by oleate[8]. Eriksson *et al.*[8] suggested that the ability of PEG-bound fatty acids to bind to erythrocytes may be due to some differences in the relative amounts of the cell membrane phospholipids, but they did not determine the $\Delta \log K$ values for the cells.

On the basis of this previous work, we used PEG 6000 monopalmitate as a hydrophobic ligand in a dextran T40–PEG 6000 phase system containing 0.15 *M* sodium chloride and 0.01 *M* sodium phosphate buffer (sodium chloride system in Table II). This composition was chosen to distribute most blood cells at the interface of the phases in the absence of PEG palmitate in order to increase the K_p values and to increase the Δ log K values for the cells. Because of this, in the absence of PEG palmitate, blood cells partition between the upper phase and the interface of dextran–PEG systems, unlike soluble materials which distribute between two phases according to their solubilities. An increase in sodium chloride concentration with a concomitant decrease in phosphate concentration to keep the overall salt concentration essentially isotonic results in a decrease in the electrostatic potential difference between the phases, because this salt partitions almost equally[5] in both upper and lower phases, while sodium phosphate is distributed unequally in the two phases (and the potential difference increase). Besides at pH 7.5, the partition experiments were also carried out at the $pH_{c.p.}$ (isoelectric point pH) for the different kinds of blood cells[6] to quench electrostatic effects and to manifest hydrophobic interactions. Under these conditions the partition of negatively charged blood cells should be determined solely by surface properties other than the charge, *e.g.*, the hydrophobic character of that part of the membranes exposed to the surroundings.

The partition coefficients, K_0 and K_p, of the four blood cells in the presence or absence of PEG palmitate are compared in Fig. 2 and Table II. These coefficients

obtained at pH 7.5 are, in general, slightly greater than those at $pH_{c.p.}$, and suggest that the electrostatic interactions between the cell surface and the phase system make a small contribution to the partition of these cells at pH 7.5. It is also evident from Fig. 2 that the two-phase systems used are suitable for observing the effect of PEG palmitate on the hydrophobic affinity partition of the blood cells.

Excellent linear plots of the $\Delta \log K$ values of platelets, granulocytes and lymphocytes at their $pH_{c.p.}$ *versus* the retention volumes of these cells on the PEG 20M–Sepharose 6B column were obtained for all three kinds of PEG palmitate fractions in the two-phase systems (see Fig. 4). A similar dependence was found for using the $\Delta \log K$ values determined in the absence of sodium chloride (Fig. 5). These dependences of the retention volumes on $\Delta \log K$ as a measure of the cell surface hydrophobicity provide direct evidence that the retention behaviour of these cells, except for erythrocytes, is mainly based on the hydrophobic interactions between the cell surfaces and the bonded PEG stationary phase and is almost independent of the electrostatic interactions between them. These results seem to be in line with previous observations[6] about the chromatographic behaviour of human blood cells on PEG–Sepharose columns. The retention volumes of lymphocytes, granulocytes and platelets at pH 7.5 and the $pH_{c.p.}$ showed marked dependences on the average molecular weight of the bonded PEG in the range of 400–20,000, increasing with increasing molecular weight.

The much higher value of $\Delta \log K$ of erythrocytes is due to the higher K_p and lower K_0 values at both pH 7.5 and 5.5 in the sodium chloride system (see Table II), and this results in an exception in the relationship between the values and the retention volumes of blood cells. Human erythrocytes, because of their low surface charge compared with those of the corresponding cells of beef, horse, lamb and pig, are little distributed in the upper phase of the dextran T500–PEG 6000 system containing sodium chloride at pH 6.8[15]. Walter[16] suggested that PEG may be able to intercalate the membrane of erythrocytes partitioned in the two-phase system. The basis for this suggestion was a correlation found between the partition coefficient of erythrocytes from different species and the ratio of their membrane polymonounsaturated fatty acids[15].

Compared with the partition in the sodium chloride system, the partition coefficients, K_0, of the blood cells in the absence of sodium chloride tend to increase slightly (Table II), possibly due to an increase in phosphate concentration from 0.01 *M* to 0.13 *M*. A marked increase in the K_0 of erythrocytes was observed at pH 7.5. The increase in the K_p value of the cells at pH 7.5 is remarkable considering the decrease in the value at pH 5.5 compared with that determined in the sodium chloride system. Phosphate is known to increase the partition of negatively charged particles in PEG-rich upper phase, as a consequence of the interaction between dextran and the anion[3]. Therefore, it is considered that the cells increased the partition in the upper phase at pH 7.5. These higher K_p values at both pH 7.5 and 5.5 led to the higher $\Delta \log K$ values of the cells.

We have shown[2] that the retention volume of human blood cells, except for platelets, on a PEG 20M–Sepharose column increased upon incorporation of sodium chloride in the eluent buffer. When an eluent such as a phosphate-buffered solution of 2% (w/w) dextran T40 containing 0.11 or 0.13 *M* sodium chloride was used, granulocytes were significantly adsorbed on the column, and lymphocytes also ad-

sorbed at the concentration of 0.13 *M*. In particular, erythrocytes were entirely adsorbed in the presence of 0.11 and 0.13 *M* sodium chloride. It should be noted that the retention volumes of human erythrocytes and platelets were also small and similar in the absence of sodium chloride (see Table I). Nevertheless, there was a marked difference between their partition coefficients and in the $\Delta \log K$ values in the dextran–PEG two-phase system. Therefore, we suppose that the more rapid elution of erythrocytes compared with the other cells by eluents containing no sodium chloride might be due to the insufficiency of the hydrophobicity of PEG 20M–Sepharose to interact with the cell surface. This could be supported by our unpublished experiments which showed that erythrocytes were not eluted from either phenyl-Sepharose CL-4B or cross-linked polyvinylpyrrolidone columns using various eluents in which sodium chloride was absent, because of the irreversible adsorption. It should be mentioned that the hydrophobicities of these two kinds of packings are much greater than that of PEG 20M–Sepharose. A detailed interpretation of the significance of the higher $\Delta \log K$ values of erythrocytes cannot be made at present. In order to understand completely the factors governing cell partition in a two-phase system, a thorough study of the cell membrane constituents is called for.

In conclusion, we have found that the retention volumes of platelets, granulocytes and lymphocytes on PEG 20M–Sepharose are directly related to $\Delta \log K$ values determined at the $pH_{c.p.}$ which are a measure of the surface hydrophobicities of these blood cells. It has been demonstrated that the surface hydrophobicities of the blood cells, except for erythrocytes, play a dominant rôle in determining the retention behaviour of these cells in this system. However, further experimentation is necessary to explain the higher $\Delta \log K$ values of erythrocytes.

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