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Transport of Organic Anions Through the Erythrocyte Membrane as K^+-Valinomycin Complexes

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Summary. K^+, Rb^+, or Cs^+ complexes of valinomycin form ion pair complexes with picric acid and trinitrobenzenesulfonate (TNBS). The formation of a picrate-K^+-valinomycin complex is supported by spectral evidence. These complexes have zero net charge and readily permeate the intact erythrocyte membrane. The K^+-valinomycin complex has been used to convert the nonpenetrating TNBS into a penetrating covalent probe, making it as useful vectorial probe to measure accessible amino groups of proteins and phospholipids on both sides of the erythrocyte membrane.

The enhanced transport of TNBS into the cell by valinomycin is dependent on external K^+ in the medium. The entry of TNBS into the cell is manifested by an increased labeling of hemoglobin and membrane phosphatidylethanolamine (PE).

Stilbeneisothiocyanatedisulfonate (SITS) and anilinonaphthalenesulfonate (ANS) inhibit both the basal and K^+-valinomycin stimulated labeling of PE and hemoglobin by TNBS. The data suggest two independent effects of ANS and SITS, one mediated by an inhibition of the anion transport protein and another by the incorporation of these hydrobic anions into the cell membrane with an increase in negative charge on the membrane which leads to an inhibition of TNBS permeation into the cell by electrostatic repulsion.

In order to determine the asymmetry of amino-phospholipids in cell membranes, two different chemical probes have been used. One probe readily penetrates the membrane and labels aminophospholipids on both sides of the membrane. The second probe does not penetrate the membrane to any significant extent and labels aminophospholipids on the exterior surface only. These two probes must have different chemical properties since one penetrates and one does not penetrate the cell. Indeed the penetrating probe is usually nonionic and hydrophobic whereas the nonpenetrating probe is anionic. This creates inherent difficulties in chemical reactivity and solubility of the probes which opens to question a direct comparison of the degree of labeling of membrane components by these two probes. If a method were developed

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to convert a nonpenetrating probe into a penetrating probe without altering its structure, the same probe could be used to label components on both sides of the membrane and circumvent the above mentioned problems. We have done this in the present paper by use of valinomycin.

Valinomycin forms complexes with K^+, Rb^+, and Cs^+ and can shuttle these cations across cell membranes and artificial lipid membranes (Pressman, 1968; Pressman, 1973; Andreoli & Tosteson, 1971). Recent work by Gunn and Tosteson (1971) has shown that the organic trinitrocresolate anion is an effective counterion to K^+-valinomycin in hydrocarbon solvents. They found that the concentration of K^+ in a solution of valinomycin in decane equilibrated with aqueous solutions of K^+ salts was greatly increased by trinitrocresol. Furthermore K^+ conductance of lipid bilayers in the presence of valinomycin was markedly enhanced by trinitrocresol. The formation of an ion pair complex of trinitrocresolate anion with K^+-valinomycin has been sustantiated by nuclear magnetic resonance studies (Davis & Tosteson, 1975).

Previous studies in our laboratory have shown that trinitrobenzene sulfonate (TNBS)[1] does not penetrate the red cell membrane to any appreciable extent at 23 °C as compared to fluorodinitrobenzene (FDNB) (Gordesky & Marinetti, 1973; Gordesky, Marinetti & Love, 1975; Marinetti & Love, 1976). Therefore, TNBS can be used as a vectorial probe for the red cell membrane and can label accessible amino groups of phospholipids and proteins on the outer surface of the erythrocyte membrane. In order to use TNBS as a vectorial probe for other cell membranes, one must be able to ascertain whether this probe penetrates the membrane. With the erythrocyte this is relatively easy to ascertain since the labeling of hemoglobin can be used to measure penetration. With other systems, such as mitochondria, mycoplasma, bacteria, and other eukaryote cells, the degree of the penetration of TNBS is more difficult to ascertain. In this paper we provide evidence that K^+-valinomycin forms a complex with TNBS which is permeable to the red cell membrane. This technique converts TNBS from an impermeable probe to a permeable probe, thereby circumventing the problems inherent in using two different probes to evaluate membrane asymmetry. It also allows the evaluation of the degree of penetration of TNBS in cells other than the erythrocytes.

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1 *Abbreviations:* TNBS – 2,4,6-trinitrobenzenesulfonic acid; FDNB – 1-fluoro-2,4-dinitrobenzene; ANS – 1-anilino-8-naphtalenesulfonic acid; SITS – acetamido-4-isothiocyanostilbene-2,2′-disulfonic acid; PE – phosphatidylethanolamine; PS – phosphatidylserine, Hb – hemoglobin.

Materials and Methods

Human erythrocytes (Red Cross Blood Bank) were washed three times with Krebs-Ringer bicarbonate buffer (KRB [2]) pH 7.4. 0.5 ml aliquots of packed cells were suspended in 19.5 ml of KRB buffer, pH 7.4. In some experiments as indicated in the text, the K^+ concentration was altered from 0–120 mM. The valinomycin and organic ions were added to the cell suspension in small aliquots (50–200 μl) of methanol. Control cell suspensions contained the same amount of methanol. Under our experimental conditions cell lysis was very small (less than 2%). The analysis of TNP-PE and TNP-hemoglobin was carried out as described previously (Gordesky *et al.*, 1975).

The following reagents were used in these experiments: valinomycin (Sigma Chemical Co.) 2,4,6-trinitrobenzenesulfonic acid (Pierce Chemical Co.), 1-anilino-8-naphthalene sulfonic acid (ANS) (Nutritional Biochemicals) acetamido-4-isothiocyanostilbene-2,2′-disulfonic acid (SITS) (Nutritional Biochemicals), benzenesulfonic and (Eastman Kodak) *m*-nitrobenzene-sulfonic and (Eastman Kodak) picric acid (Merck & Co., Inc.).

Results

Our previous studies on TNBS labeling of erythrocytes were done at pH 8.5 in 120 mM NaCl – 40 mM $NaHCO_3$ buffer at 23 °C for 1 hr. Under these conditions, saturation of accessible amino groups of PE and membrane protein located on the outside surface of the cell occurred at a concentration of 1–2 mM TNBS (Marinetti & Love, 1976). Moreover, under these conditions PS and hemoglobin are labeled only to an extremely small extent. In these present experiments we chose to work at pH 7.4 in a more physiological buffer and therefore had to establish the optimal concentration of TNBS and time of reaction to allow interaction of the probe only with amino groups accessible on the outer surface of the cell membrane. We wished to minimize penetration of TNBS into the cell and had to select conditions which represented a compromise between saturation of available amino groups and minimal perturbation of the cell (no hemolysis and minimal loss of cell K^+).

When cells are reacted for 1 hr at 23 °C at pH 7.4 with varying amounts of TNBS (1–6 mM) a nonlinear curve (Fig. 1) is obtained for the extent of reaction of membrane PE. At 1 mM TNBS only 1.5% of the total membrane PE has reacted, whereas at 6 mM TNBS only 3.2% of the total PE has reacted. If the profile in Fig. 1 is corrected by subtraction of the linear portion of the curve between 2–6 mM TNBS, a saturation

2 The KRB buffer pH 7.4 contains (in mM): 119, NaCl; 4.8, KCl; 1.2, $CaCl_2$; 1.2, NaH_2PO_4; 1.2, $MgSO_4$; and 25, $NaHCO_3$. In experiments where K^+ was varied, the NaCl was replaced by KCl or the KCl was omitted. The same was done when Rb^+ and Cs^+ were varied (Figs. 4, 5 and 6).

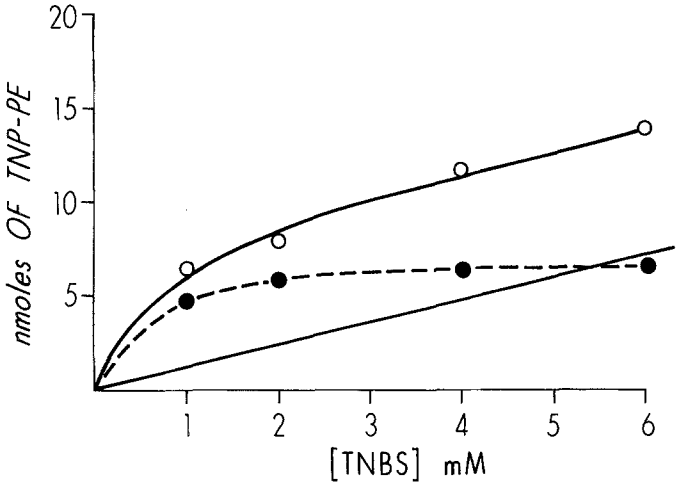

Fig. 1. Extent of reaction of PE with varying concentrations of TNBS. 0.4 ml of washed packed cells were suspended in 20 ml of KRB buffer pH 7.4 and reacted for 1 hr at 23 °C with TNBS at concentrations varying from 1–10 mM. The cells were removed by centrifugation, washed, lysed, and extracted with chloroform/methanol 1:1. The labeled TNP – PE and unreacted PE were separated and analyzed by thin layer chromatography as described previously (Gordesky, Marinetti & Love, 1975). TNP – PE was analyzed by its absorbance at 337 nm. The unreacted PE was eluted, digested with perchlorid acid, and analyzed for phosphate

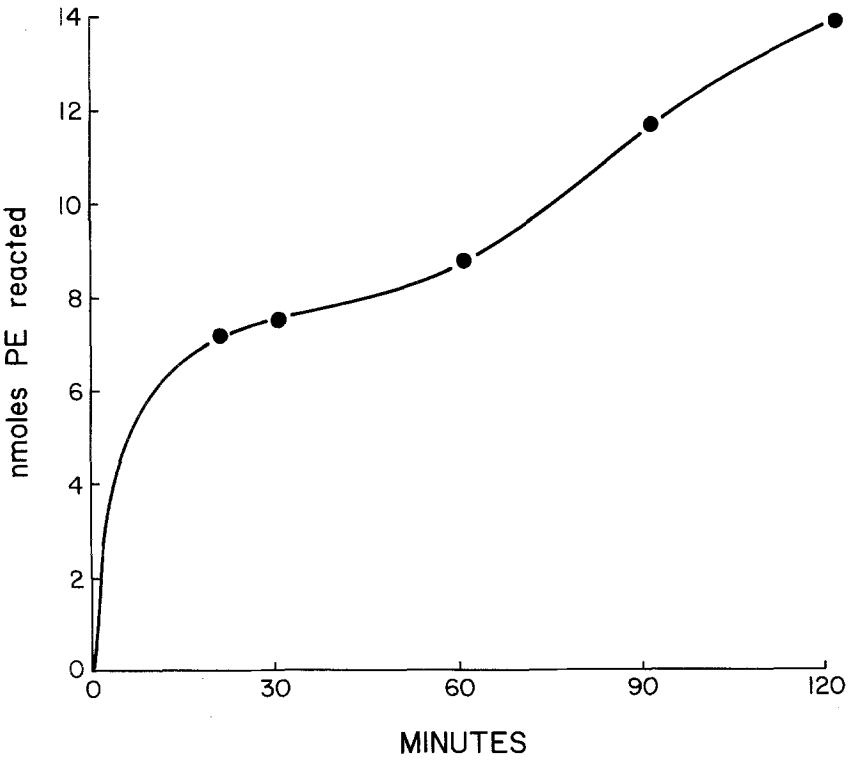

Fig. 2. Time course of reaction of PE with TNBS. 0.4 ml of washed packed cells were suspended in 20 ml of KRB buffer pH 7.4 and reacted for different times at 23 °C with 2 mM TNBS. The extent of reaction of PE was determined as explained in Fig. 1

profile results as shown by the dashed curve. This profile indicates that only 1.6% of the total PE molecules are accessible on the outer surface of the membrane under these conditions if one assumes that the linear portion represents slow penetration into the cell. We showed previously that pH 8.5 the extent of reaction of PE in intact cells is about 5% under conditions of 2 mM TNBS for 1 hr (Marinetti & Love, 1976).

The time course of reaction of 2 mM TNBS for 30 min in KRB buffer pH 7.4 is shown in Fig. 2. An initial plateau occurs at 30 min followed by a progressive linear increase between 1 and 2 hr. This latter may be due in part to a very slow penetration of TNBS into the cell as the cell is perturbed by the probe. The plateau at 30 min occurs when 1.7% of the total membrane PE has reacted and agrees with the corrected value of 1.6% in Fig. 1. After 2 hr the extent of reaction of PE is 3.2%. The labeling of hemoglobin and PS under these conditions is too small to measure accurately.

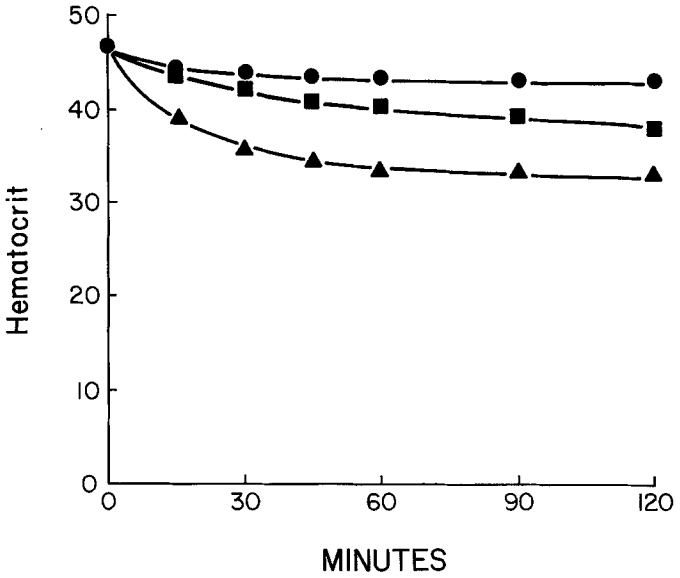

Fig. 3. Effect of valinomycin on the cell volume of erythrocytes. 0.5 ml of washed packed cells were suspended in 0.5 ml of KRB buffer pH 7.4 containing 1 μM valinomycin. Control cells were suspended in buffer alone. The valinomycin was added in 5 μl of methanol. Control cells also contained 5 μl of methanol. The cells were incubated either at 23 or 37°C for different times, then centrifuged in hematocrit capillary tubes on a clinical centrifuge fitted with a horizontal head. Hematocrits were determined by use of a MSE micro-hematocrit reader. ●=control, 23° and 37°; ■=1 μM valinomycin, 23°C; ▲=1 μM valinomycin, 37°C

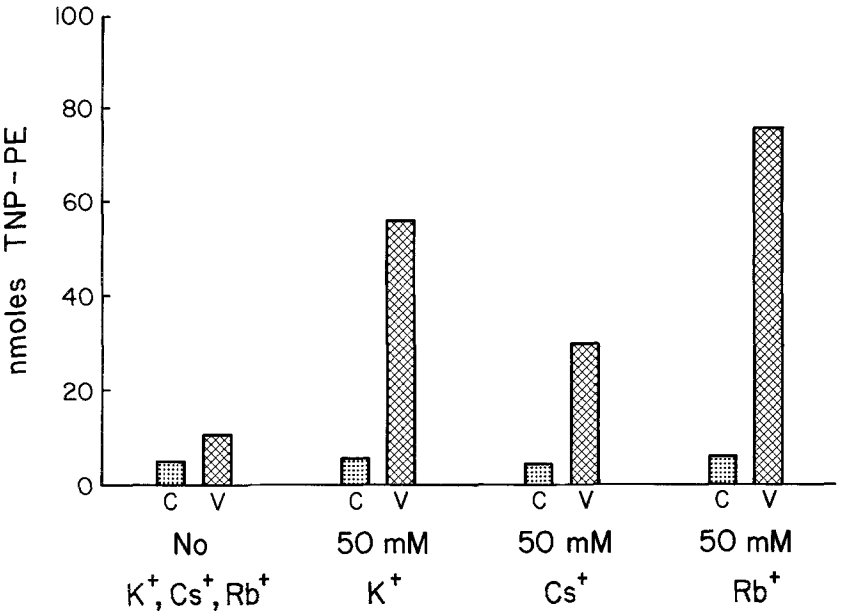

Fig. 4. Effect of K^+, Rb^+ and Cs^+ on the valinomycin enhanced labeling of PE with TNBS. 0.5 ml of washed packed cells were incubated for 30 min at 23 °C in 20 ml of KRB buffer pH 7.4 containing 1 mM TNBS, 1 μM valinomycin, and with different amounts of KCl, CsCl or RbCl. A fraction of the NaCl in the KRB buffer was replaced by KCl, CsCl, and RbCl to make each salt 50 mM. The valinomycin was added in 5 μl of methanol. The TNP – PE was analyzed as described in Fig. 1

Since K^+, Rb^+, and Cs^+ form complexes with valinomycin, we examined the effect of these ions on the valinomycin stimulation of the labeling of PE by TNBS. The results are shown in Fig. 4. At 50 mM cation concentration the effectiveness of the monovalent cations was $Rb^+ > K^+ > Cs^+$. The concentration dependency of K^+ and Rb^+ are shown in Figs. 5 and 6. The enhanced labeling of PE and hemoglobin by TNBS are dependent on added K^+ or Rb^+ in a nonlinear fashion. The labeling of hemoglobin reaches near saturation at 120 mM K^+ in the presence of 1 μM valinomycin, whereas the labeling of internal PE does not reach saturation under these conditions. The enhanced labeling of hemoglobin by K^+-valinomycin shows that the transport of TNBS into the cell is stimulated by this complex.

The above effects of valinomycin and cations on the labeling of PE and hemoglobin suggest that positively charged complexes of valinomycin (V) with the cation (M^+) lead to a ternary complex, $(M-V)^+ - TNBS^-$ which is able to permeate the red cell membrane. When the

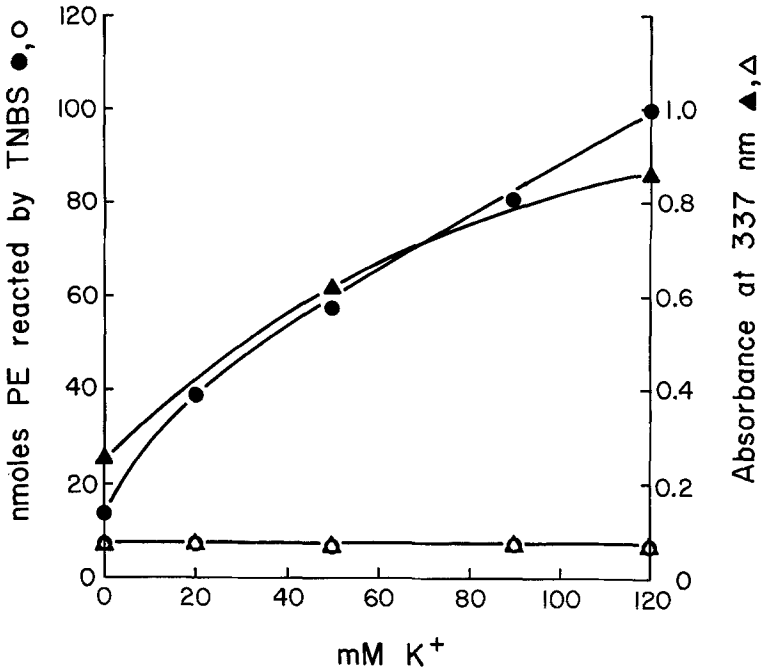

Fig. 5. Effect of K^+ concentration on the valinomycin enhanced labeling of PE and hemoglobin by TNBS. 0.5 ml of washed packed cells were suspended in 20 ml of KRB buffer pH 7.4 in which the NaCl was replaced by KCl to give 0, 20, 50, 90 and 120 mM K^+. Cells were incubated with and without 1 μM valinomycin and with 1 mM TNBS for 1 hr at 23°C. The labeling of PE and hemoglobin were determined as described previously (Gordesky *et al.* (1975). ●=TNP−PE, 1 μM valinomycin; ○=TNP−PE, no valinomycin; ▲=TNP-globin, 1 μM valinomycin; △=TNP-globin, no valinomycin

concentration of K^+ or Rb^+ is 50–112 mM, the valinomycin-induced transport of TNBS occurs with no significant change in hematocrit or hemolysis. Hence the cell size and the cell membrane surface area is not appreciably perturbed by these agents under these conditions. If K^+ is omitted from the buffer, valinomycin will induce a net loss of K^+ from the cell and cause a decrease in hematocrit as seen in Fig. 3.

Further evidence for the formation of a ternary complex of TNBS with K^+-valinomycin was obtained by use of competitive organic anions. These results are shown in Table 1. These organic anions such as ANS, picric acid, benzenesulfonic acid and *m*-nitrobenzenesulfonic acid do not react covalently with functional groups of membrane proteins or PE. Under our experimental conditions, SITS reacts covalently with membrane proteins or PE only to a very small extent, far less than does TNBS.

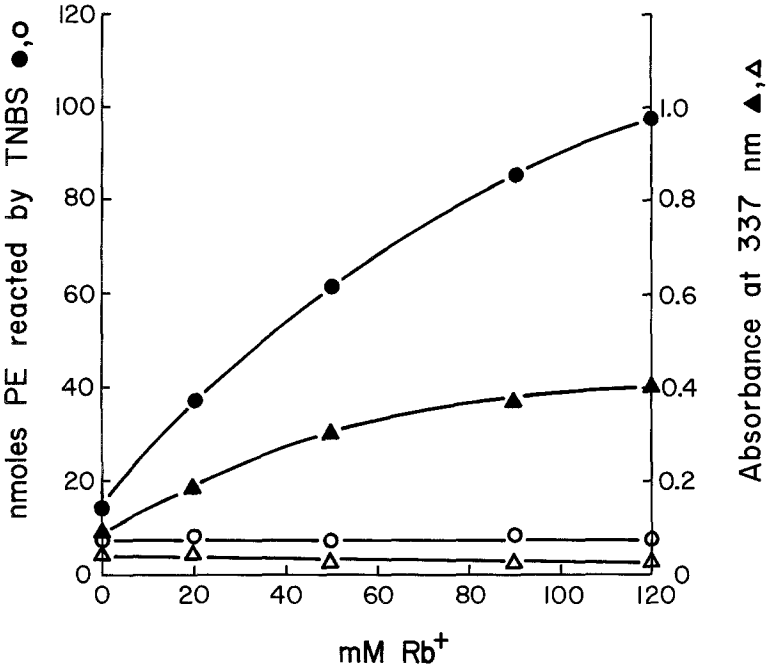

Fig. 6. Effect of Rb^+ concentration on the valinomycin-enhanced labeling of PE and hemoglobin by TNBS. The reaction of cells was carried out as given in Fig. 5, except Rb^+ was substituted for K^+. *See* Fig. 5 for symbols

Table 1. Effect of organic anions on the valinomycin-induced labeling of phosphatidylethanolamine and hemoglobin of intact erythrocytes by trinitrobenzenesulfonate

Organic anion	Phosphatidyl-ethanolamine		Hemoglobin	
	Control	Valinomycin	Control	Valinomycin
Experiment I	nmoles		Absorbance	
None	6.6 ± 0.18	51.9 ± 1.8	0.039 ± 0.004	0.35 ± 0.014
SITS	4.8 ± 0.48	37.8 ± 1.1	0.030 ± 0.001	0.320 ± 0.004
ANS	2.8 ± 0.24	21.7 ± 1.6	0.016 ± 0.009	0.375 ± 0.014
Experiment II				
None	9.2	64.2	0.038	0.317
Picric acid	5.4	23.4	0.026	0.188
Benzenesulfonic acid	8.9	64.1	0.034	0.332
m-nitrobenzenesulfonic acid	9.5	56	0.043	0.310

The reactions were carried out with 0.5 ml packed human erythrocytes plus 19.5 ml of Krebs-Ringer-bicarbonate buffer pH 7.4. The buffer had 50 mM K^+. The valinomycin was added in 50 µl of methanol to give a final valinomycin concentration of 1 µM. The organic ions and TNBS were added in 200 µl of methanol to give a final concentration of 1 mM for the organic anions and 2 mM for TNBS. Control systems contained the same amount of cells, buffer, and methanol. The reaction time for TNBS was 30 min at 23°C. In experiment *I*, the values are the mean ± SD of triplicate analyses of three experiments. In experiment *II*, the values are the average of duplicate analyses.

The results in Table 1 show that organic anions can have several effects depending on whether they bind to the lipids or proteins of the membrane of whether they compete with TNBS for K^+-valinomycin. ANS inhibits both the basal and valinomycin induced labeling of PE by TNBS. On the other hand, it inhibits the basal labeling of hemoglobin by TNBS but enhances the labeling of hemoglobin when valinomycin is present. These results can be explained by ANS binding to the lipid bilayer of the membrane and protecting membrane PE from TNBS, possibly by electrostatic screening. Fortes and Hoffman (1971) have suggested that ANS binds to the red cell membrane and alters its surface charge. The increased negative charge on the membrane due to ANS binding may be expected to decrease the penetration of TNBS into the cell where it can label hemoglobin. However, since the ANS is bound in

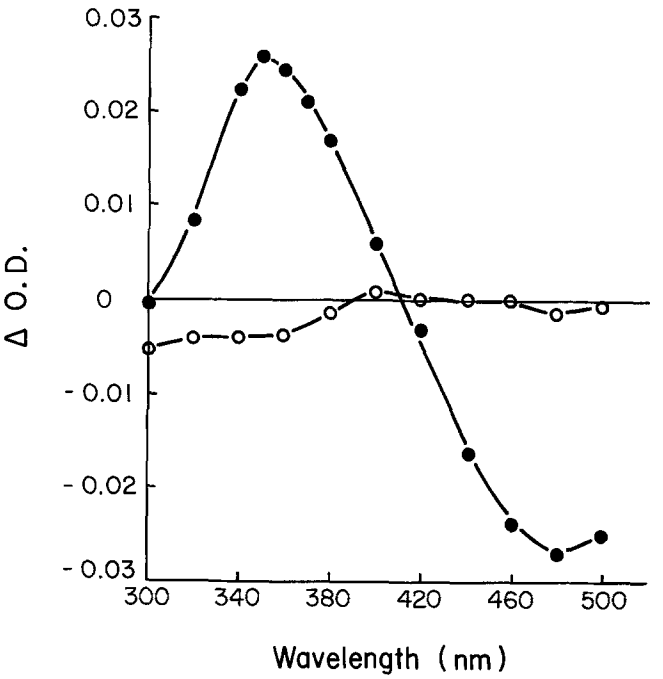

Fig. 7. Difference spectrum showing complex ion formation of picric acid with K^+ valinomycin. Two double sector matched cells were used to determine the difference spectrum. Compartment *A* of cell *1* contained 20 μM picric acid in KRB buffer pH 7.4, and compartment *B* contained 10 μM valinomycin in KRB buffer pH 7.4. Compartment *A* of cell 2 contained 20 μM picric acid and 10 μM valinomycin, whereas compartment *B* contained KRB buffer pH 7.4. All buffers contained 2.5% methanol and 50 mM KCl. The difference spectrum was obtained by reading the cell *1* against cell *2* and is shown by the solid circles (●). A control experiment in which no KCl was included in the buffer is shown by the open circles (○). The spectra were obtained on a Gilford spectrophotometer

the lipid bilayer, it also can by electrostatic screening protect some PE molecules from reacting with TNBS.

The results with SITS are quite different. SITS is a polar, anionic, nonpenetrating probe and binds quite specifically to band 3, the anion transport protein (Rothstein, Cabantchik & Knauf, 1976). SITS causes a decrease in the basal labeling of both PE and hemoglobin by TNBS but it also decreases the labeling of these components in the presence of valinomycin. SITS is not as effective as ANS since it probably does not bind to the lipid bilayer as much as the more hydrophobic ANS. The major effect of SITS appears to be its competition with TNBS for K^+-valinomycin. If a very small amount of TNBS can penetrate the cell membrane via the anion transport protein, SITS can block this transport since it is known to bind quite selectively to the anion transport protein

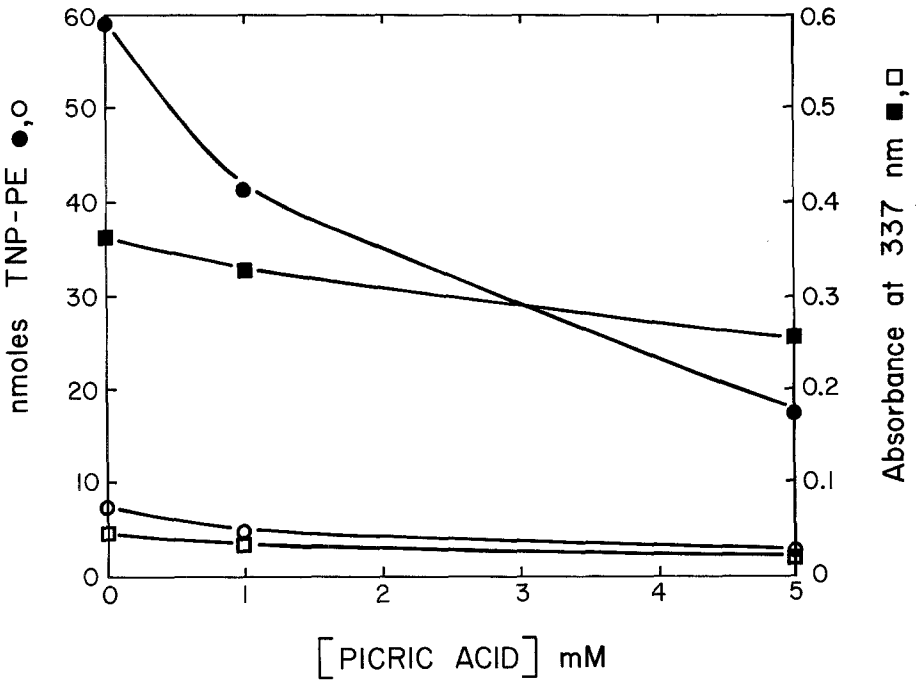

Fig. 8. The inhibition by picric acid of the valinomycin-enhanced labeling of PE and hemoglobin by TNBS. 0.5 ml of washed packed cells were suspended in 20 ml of KRB buffer pH 7.4 containing 50 mM KCl and 1 mM TNBS. Experimental cells were treated with 1 μM valinomycin. Control cells were not exposed to valinomycin. All cells were then incubated at 23°C with varying amounts of picric acid. The labeling of PE and hemoglobin were determined as described previously (Gordesky *et al.*, 1975). ●=TNP-PE, 1 mM valinomycin; ○=TNP-PE, no valinomycin; ■=TNP-globin, 1 μM valinomycin; □=TNP-globin, no valinomycin

and inhibit the transport of Cl^- and $SO_4^=$ (Fortes & Hoffman, 1971; Fortes & Hoffman, 1974; Rothstein *et al.*, 1976).

Picric acid was found to be a strong inhibitor of the TNBS labeling of both PE and hemoglobin. Picric acid is a trinitrophenol which has a pK ~ 2.8. It readily penetrates the cell membrane. We find that picric acid forms a complex with K^+-valinomycin as shown by the difference spectrum in Fig. 7. In this regard picric acid is similar to the trinitrocresol (Gunn & Tosteson, 1971). Since picric acid forms a complex with K^+-valinomycin, it can inhibit the valinomycin-induced transport of TNBS into the cell. In this manner it inhibits the TNBS labeling of PE and hemoglobin. Organic anions such as benzenesulfonic acid and *m*-nitrobenzenesulfonic acid may not form stable complexes with K^+-valinomycin and have little effect of the valinomycin-induced labeling of PE and hemoglobin by TNBS.

A more detailed study of the concentration dependency of picric acid on the valinomycin-induced labeling of PE and hemoglobin by TNBS is shown in Fig. 8. The effect of picric acid is greater on the labeling of PE than on the labeling of hemoglobin both with and without valinomycin. Thus, picric acid appears to have two effects, one mediated by its ability to form a complex with K^+-valinomycin (this leads to inhibition of labeling of both PE and hemoglobin) and one mediated by its solubility in the membrane lipid bilayer (this leads to a decrease in labeling only of PE).

Discussion

Under appropriate conditions of temperature and buffer composition, TNBS can be used as a vectorial probe to label amino groups of proteins and phospholipids on the outer surface of the erythrocyte membrane. It would be desirable to use this same probe to label amino groups on the interior surface of the erythrocyte membrane in intact cells under conditions where minimal perturbation of the cell membrane occurs. This was accomplished in the present studies by the addition of K^+-valinomycin to the system and forming a TNBS – K^+-valinomycin complex which is a sufficiently stable complex having zero net charge which permeates the cell membrane. The idea to use K^+-valinomycin for this purpose was based on the work of Davis and Tosteson (1975) who reported complex formation of trinitrocresolate ion with K^+-valinomycin.

The ability of K^+-valinomycin to carry TNBS into the erythrocyte was based on two observations, namely the enhanced labeling of hemoglobin and the enhanced labeling of phosphatidylethanolamine. Direct spectral evidence for a TNBS–K^+-valinomycin complex was difficult to obtain due to the low absorbance of TNBS. We therefore used picric acid as a model anion for this study and show by difference spectral analysis the formation of a picrate-K^+-valinomycin complex and also show that picrate inhibits the TNBS labeling of PE and hemoglobin in intact red cells.

The enhanced TNBS labeling of PE and hemoglobin by K^+-valinomycin was inhibited by SITS, ANS, and picric acid but not by benzenesulfonic acid and very little by *m*-nitrobenzenesulfonic acid. Both SITS and ANS are known to inhibit anion transport in erythrocytes (Fortes & Hoffman, 1974; Rothstein *et al.*, 1976), whereas ANS is postulated by Fortes and Hoffman to have two effects, one mediated by inhibiting the anion transport protein and another by modifying the surface charge on the membrane. Our results are consistent with these ideas and indicate that K^+-valinomycin stimulated TNBS transport via two pathways: one via the lipid bilayer and one via the anion transport system. Both SITS and ANS inhibit the very small basal TNBS labeling of PE and hemoglobin. ANS is more effective than SITS, indicating that there is a very small penetration of TNBS into the cell mediated through the lipid bilayer. ANS is believed to insert itself into the bilayer more effectively than SITS and produce an electrostatic screening to the penetration of TNBS. This type of effect is consistent with the proposal of Fortes and Hoffman, 1974.

In our system the effect of K^+-valinomycin in enhancing TNBS permeability occurs under conditions where no appreciable change occurs in cell volume as determined by measuring the hematocrit. However, this is so only if the K^+ concentration is between 50–150 mM. If K^+ is omitted, then the cells undergo shrinkage when treated with valinomycin (as seen in Fig. 3).

Both Rb^+ and Cs^+ can replace K^+ as cations in inducing the valinomycin enhanced labeling of PE by TNBS. In our system Rb^+ is most effective and Cs^+ least effective. This order of effectiveness of these cations is enhancing TNBS entry into the cell is the same as that for the valinomycin-dependent transport of monovalent cations (Pressman, 1968, 1973).

In our system both Rb^+ and K^+ complexes of valinomycin show a plateau in the TNBS labeling of hemoglobin, whereas the labeling of PE

has not reached saturation. This indicates that once TNBS has been transported into the cell as a valinomycin-cation ion pair complex it more readily reacts with hemoglobin than PE. This may be due in large part to the high concentration of hemoglobin inside the cell. However, when picric acid is added to the system containing K^+-valinomycin and TNBS, the labeling of PE is inhibited more than the labeling of hemoglobin. This suggests that picric acid competes with TNBS for K^+-valinomycin and that picric acid concentrates in the membrane and protects some PE molecules from reacting with TNBS.

In these studies we observed that valinomycin induced a net loss of K^+ and inorganic phosphate from the red cells but also led to a net uptake of Na^+ from the medium. It is hoped to publish the results of these experiments at a later time.

References

Andreoli, T.E., Tosteson, D.C. 1971. The effect of valinomycin on the electrical properties of solutions of red cell lipids in *n*-decane. *J. Gen. Physiol.* **57**:526

Davis, D.G., Tosteson, D.C. 1975. Nuclear magnetic resonance studies on the interactions of anions and solvent with cation complexes of valinomycin. *Biochemistry* **14**:3962

Fortes, P.A.G., Hoffman, J.F. 1971. Interactions of the fluorescent anion 1-anilino-8-naphthalene sulfonate with membrane charges in human red cell ghosts. *J. Membrane Biol.* **5**:154

Fortes, P.A.G., Hoffman, S.F. 1974. The interaction of fluorescent probes with anion permeability pathways of human red cells. *J. Membrane Biol.* **16**:79

Gordesky, S.E., Marinetti, G.V. 1973. The asymmetric arrangement of phospholipids in the human erythrocyte membrane. *Biochem. Biophys. Res. Commun.* **50**:1027

Gordesky, S.E., Marinetti, G.V., Love, R. 1975. The reaction of chemical probes with the erythrocyte membrane. *J. Membrane Biol.* **20**:111

Gunn, R.B., Tosteson, D.C. 1971. The effect of 2,4,6-trinitro-*m*-cresol on cation and anion transport in sheep red blood cells. *J. Gen. Physiol.* **57**:593

Marinetti, G.V., Love, R. 1976. Differential reaction of cell membrane phospholipids and proteins with chemical probes. *Chem. Phys. Lipids* **16**:239

Pressman, B.C. 1968. Ionophorous antibiotics as models for biological transport. *Fed. Proc.* **27**:1283

Pressman, B.C. 1973. Properties of ionophores with broad range cation selectivity. *Fed. Proc.* **32**:1698

Rothstein, A., Cabantchik, Z.I., Knauf, P. 1976. The mechanism of anion transport in red blood cells: Role of membrane proteins. *Fed. Proc.* **35**:3