The accelerated turbidity change induced by oligomycin —which is known to inhibit transport ATPase—suggests that this drug promotes formation of the stable complex (shown above) and, hence, stops net ion flow across the membrane.

The model proposed for cation exchange in myelin can be compared with the erythrocyte cation pump[3]. Our results suggest that the outer surface of myelin can react with external ATP and ADP. It is well known that ion pumping in the red cell membrane is not affected by external adenine nucleotides; ATP on the outside is not hydrolysed by the cell membrane. In our myelin model potassium binding is linked with ATP consumption, while in the erythrocyte, internal sodium binding is associated with ATP consumption[3].

The particular significance of this myelin function may best be explained by considering the paranodal region of the myelinated nerve fibre. At this point the terminal myelin loops dip in and impinge on the axon; this battery of tight junctions would permit rapid local changes in Na^+ and K^+ binding. The nucleotides required could be provided by the paranodal apparatus[4]. Such a mechanism could be important in regenerating the action potential at the node, in accord with the hypothesis of saltatory conduction.

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Binding of Tritiated Digoxin to Human Red Cell Ghosts

The binding of cardiotonic glycosides to $(Na^+ + K^+)$-activated ATPase preparations from heart muscle, cat brain and *Electrophorus* electric organ requires certain specific conditions[1,2]. The data are consistent with the same quantitative relationship between glycoside binding, Na^+-dependent phosphorylation and $(Na^+ + K^+)$-activated ATP hydrolysis in all these tissues, although they differ considerably in their specific activities. Erythrocytes are another tissue from which $(Na^+ + K^+)$-activated ATPase preparations of low specific activity can easily be made, and which can also be used for studies of glycoside binding. Our experiments show that there is a similar binding of specifically labelled digoxin to a red cell ghost preparation.

Red cell ghosts were prepared from fresh haematologically normal human blood by a modification of the Dodge method[3]. The cells were rapidly frozen and thawed twice to ensure fragmentation. The preparation was assayed for ATPase by incubating at 37° C for 30 min in the presence of 1·5 mM *tris* ATP, 1·6 mM $MgCl_2$, 0·1 mM EDTA, 0·2 mM cysteine, 10 mM *tris* pH 7·4, and either 140 mM NaCl, 12·5 mM KCl or 152·5 mM NaCl. The reaction was stopped with ice cold trichloracetic acid, added to a final concentration of 5 per cent, and the inorganic phosphate released was assayed by a modification of Weil-Malherbe and Green's method[4]. The trichloracetic acid precipitate was redissolved in 3 per cent sodium hydroxide and assayed for protein by a modified biuret method. Binding of digoxin was measured by incubating the enzyme (~2 mg in 1 ml.) with labelled glycoside (usually 10^{-6} M) at 37° C for 30 min, diluting with fifty volumes of unlabelled suspending medium, and spinning and washing twice (20,000*g*; 20 min). The precipitate was dissolved in 1 ml. of Nuclear Chicago solubilizer, mixed with scintillator, and counted in a Packard 'Tricarb' spectrometer after being stored in the dark for 24 h. Six replicates were usually used for each set of conditions; control tubes were either preincubated with 10^{-3} M ouabain, or ATP and Mg^{2+} were omitted.

Specifically labelled digoxin tritiated in the 12-α position was supplied by Dr J. Rutschmann of Sandoz A.G., Basle. This preparation was purer than generally labelled preparations[5] although it had a lower specific activity.

Digoxin binding was found to require the presence of ATP, Mg^{2+} and Na^+. It was inhibited by addition of 10^{-3} M ouabain or digoxin, and by 50 mM K^+ for digoxin concentrations of less than 10^{-7} M. Binding was rapid with digoxin concentrations greater than 10^{-7} M, all labelling having occurred within 5 min. The enzyme–digoxin complex was stable to washing in 150 mM KCl, 10^{-3} M ouabain or digoxin, but the digoxin was removed by precipitation with trichloracetic acid or ethanol, or treatment with 6 M urea. Table 1 summarizes the digoxin binding data in conditions for maximum labelling. If it is assumed that one digoxin molecule binds to one enzyme active site, that all the sites are labelled and that in the conditions of the experiment all the labelling is specific, it is possible to calculate a catalytic centre activity for the ATPase which, from the present data, gives a value of 10,500 min^{-1}. Albers *et al.*[1] calculated catalytic centre activities for three ATPase preparations, from both generally labelled ouabain binding and ^{32}P incorporation experiments, obtaining values in the range 5,000–15,000 min^{-1}, and a correlation of 0·5–1 between glycoside binding and phosphorylation. Bader *et al.*[6] have measured ^{32}P-phosphorylation in a human erythrocyte preparation, giving a catalytic centre activity of 12,100 min^{-1}. This is in good agreement with the present figure from specifically labelled digoxin binding, giving a ratio close to unity.

Table 1

No. of experiments	$(Na^+ + K^+)$-activated ATPase activity (μmoles/mg protein/h ± *S.E.*)	Bound digoxin (pmoles/mg protein ± *S.E.*)
10	0·25 ± 0·06	0·39 ± 0·08

The red cell ghosts used in these experiments contained 0·8 pg of protein per cell, which leads to an estimate of about 200 molecules of digoxin bound per cell. Because the ATPase is a membrane-bound complex, its behaviour in a fragmented ghost, where the ionic asymmetry of the intact cell is destroyed, may not be exactly the same as it is *in vivo*, but the present estimate gives a somewhat smaller number of sites than some previous ones[7,8], though it is in approximate agreement with recent work of Hoffman and Ingram[9] and of ourselves on intact human erythrocytes.

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