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CHANGES IN CARDIAC GLYCOSIDE RECEPTOR SITES, ^{86}RUBIDIUM UPTAKE AND INTRACELLULAR SODIUM CONCENTRATIONS IN THE ERYTHROCYTES OF PATIENTS RECEIVING DIGOXIN DURING THE EARLY PHASES OF TREATMENT OF CARDIAC FAILURE IN REGULAR RHYTHM AND OF ATRIAL FIBRILLATION

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1 Measurements of the binding of 12-α-[^{3}H]-digoxin to the membranes of intact erythrocytes, erythrocytic ^{86}rubidium uptake and intraerythrocytic sodium concentrations have been made in the red cells of patients receiving digoxin in the short-term for atrial fibrillation or cardiac failure in regular rhythm.

2 During the first few days of treatment [^{3}H]-digoxin binding and ^{86}rubidium uptake fall and intraerythrocytic sodium concentrations rise.

3 Subsequently parallel fluctuations occur in [^{3}H]-digoxin binding and ^{86}rubidium uptake but not in intraerythrocytic sodium concentrations and the significance of the fluctuations is discussed.

4 The values of all three measurements correlate significantly with the response of the heart in sinus rhythm as measured by QS_2I.

5 Plasma digoxin concentrations do not correlate with QS_2I.

Introduction

There is uncertainty about the relationship between the plasma concentration of digoxin and its therapeutic (as opposed to its toxic) effects (Aronson, 1975). The problem stems from the complex relationships likely to exist between the rise and fall of the plasma concentration with time following an oral dose, the subsequent pharmacological effect and the translation of that effect into a therapeutic response. We have therefore examined the effects which digoxin therapy has on various biochemical pharmacological aspects of tissue function as exemplified by the patient's red cells and have compared these pharmacodynamic effects with, on the one hand, the plasma concentration of digoxin and, on the other, the cardiac response.

Cardiac glycosides are known to inhibit cation pumping across cell membranes (Schatzmann, 1953) and this inhibition is thought to be consequent upon binding to and inhibition of Na^+,K^+-ATPase (Hoffman, 1969). It is at present uncertain whether or not Na^+,K^+-ATPase is the pharmacological receptor for digitalis (Schwartz, 1976) but the enzyme is present in man in a great variety of tissues other than cardiac muscle, including erythrocytes. The erythrocyte, therefore, provides a readily accessible source of a tissue which might constitute a peripheral marker of the effects of digitalis on cardiac Na^+,K^+-ATPase. Thus, binding of digoxin to erythrocytes during treatment might be expected to result in diminished cation flux and hence altered intraerythrocytic cation concentrations. Furthermore, the presence of digoxin bound to the erythrocyte membrane would be expected to diminish the capacity of the membrane to bind [^{3}H]-digoxin *in vitro*.

In a previous study ^{86}rubidium (^{86}Rb) uptake by the erythrocytes of patients receiving digoxin for the treatment of atrial fibrillation was inhibited compared with pre-treatment values. The extent of this inhibition correlated well with the cardiac response to digoxin in those patients (Aronson, Grahame-Smith, Hallis, Hibble & Wigley, 1977). We have now extended these studies to other patients both in atrial

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fibrillation and with cardiac failure in sinus rhythm. Furthermore we have measured patients' intra-erythrocytic cation concentrations and the capacity of their erythrocyte membranes to bind 12-α-[^{3}H]-digoxin *in vitro* during the early stages of digoxin therapy. The characteristics of this binding in normal subjects have already been described (Ford, Aronson, Grahame-Smith & Rose, 1979).

Methods

Patients

All patients received digoxin (Lanoxin, Wellcome) tablets for the treatment of cardiac failure in sinus rhythm (Group A) or for atrial fibrillation or flutter (Group B). A summary of some relevant clinical details is presented in Table 1.

Patients were given a loading dose of between 12 and 20 μg/kg in divided doses over 12–24 h and thereafter a daily maintenance dose calculated as previously described (Aronson & Grahame-Smith, 1976). One patient had aortic regurgitation and one was receiving a β-adrenergic receptor blocker; these patients have been excluded from the statistical analysis of the data concerning systolic time intervals.

Plasma digoxin concentrations

Plasma digoxin concentrations were measured by radioimmunoassay using 12-α-[^{3}H]-digoxin as radio-ligand (Smith, Butler & Haber, 1969) in samples taken at least 6 h after the previous dose of digoxin.

Erythrocytic [^{3}H]-digoxin binding, ^{86}rubidium uptake and intraerythrocytic sodium concentrations

Venous blood was collected into tubes containing lithium heparin, the plasma separated and the buffy coat discarded. The erythrocytes were washed three times in 112 mM magnesium chloride (pH 7.4) at 4°C by alternate centrifugation at 1500 *g* and re-suspension. The final centrifugation was continued for 15 min in order to pack the cells as completely as possible (mean haematocrit in 25 samples after the removal of the last supernatant was 96.8 ± 1.9%).

Erythrocytic [^{3}H]-digoxin binding was performed as previously described (Ford *et al.*, 1979) at a digoxin concentration of 100 ng/ml. It has been previously shown that the [^{3}H]-digoxin binding measured in this way offers a good prediction of the theoretical maximum binding as calculated from Scatchard plots. No account was taken of the binding which occurs in the presence of excess (10^{-3} M) ouabain and which amounts to 13(±7)% of the radioactivity bound at a digoxin concentration of 100 ng/ml in the absence of ouabain.

^{86}Rb uptake was measured as previously described (Aronson *et al.*, 1977).

Intraerythrocytic sodium and potassium concentrations were measured by flame photometry after haemolysis of the washed, packed red cells by dilution in distilled water. No correction was made for trapped magnesium chloride in the packed red cells which would amount to no more than 2% (Beilin, Knight, Munro-Faure & Anderson, 1966).

Measurement of the cardiac response to digoxin

The response to digoxin was assessed in patients with atrial dysrhythmias by counting the ventricular rate or by observing conversion to sinus rhythm.

In patients in sinus rhythm serial estimations of the systolic time intervals were made using a Schwarzer C3000 recorder at a paper speed of 100 mm/s. Simultaneous recordings were made of Lead II of the conventional electrocardiogram, of a phono-cardiogram recorded at the cardiac apex or lower left sternal edge, and of the carotid pulse wave. The systolic time intervals (QS_2, total electro-mechanical systole; LVET, left ventricular ejection time; PEP, pre-ejection period) were calculated as described by Weissler, Lewis & Leighton (1972). Corrections for heart rate were made on the basis of the regressions given by those authors for heart rates between 50 and 110 beats/min and shown to be applicable to heart rates as high as 140 beats/min by Brubakk & Overskeid (1976). The corrections for heart rate on

Table 1 Summary of patients' clinical details

	Number of patients	Age (years) (mean, range)	Sex M	Sex F	Plasma Na^+ (mmol/l)	Plasma K^+ (mmol/l)	Plasma HCO_3^- (mmol/l)	Plasma Creat (mmol/l)	Plasma Hb (g/100 ml)	Plasma MCV (fl)
Sinus rhythm	21	62 (46–82)	18	3	138±3.5	4.1±0.5	27 ±3.9	98 ±6.1	13.4±2.3	86±5
Atrial fibrillation	10	68 (51–94)	7	3	135±3.5	4.1±0.8	27.5±5.8	145.6±154	13.9±1.6	84±4.5

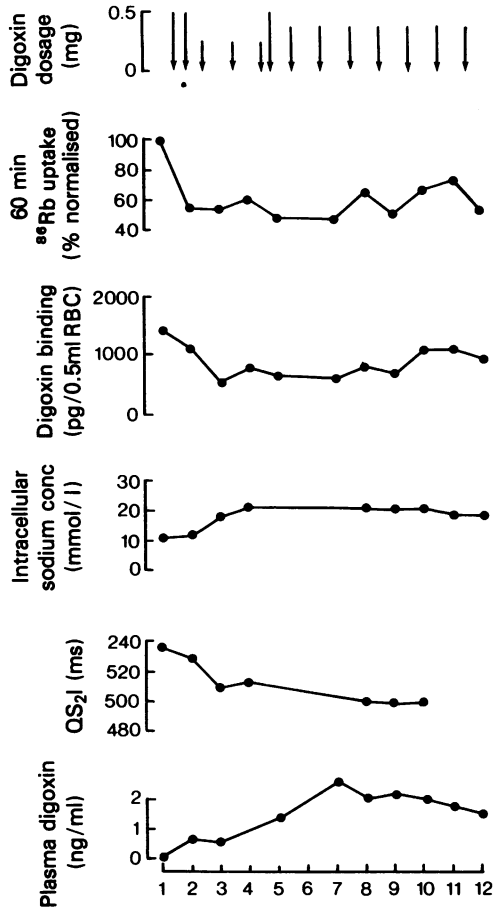

Figure 1 The time-course of changes in ^{86}Rb uptake, [^{3}H]-digoxin binding, intra-erythrocytic sodium concentrations, QS_2I and plasma digoxin concentrations following administration of digoxin.

the basis of these regressions have been shown to be unaffected by digitalis (Forester, Lewis, Weissler & Wilke, 1974). The corrected values are termed systolic time interval indices (e.g. QS_2I). For each patient, all recordings were made at the same time of day, with the patient in the same position and with the breath held in expiration.

Statistical analysis was carried out as described in Snedecor & Cochran (1967). Results are expressed as mean ± 1 s.d.

Results

Effects of digoxin therapy on erythrocytic [^{3}H]-digoxin binding, ^{86}rubidium uptake, intraerythrocytic sodium concentrations, systolic time intervals and heart rate in atrial dysrhythmias

Illustrative case. Following the administration of digoxin, changes occured in [^{3}H]-digoxin binding and erythrocytic ^{86}Rb uptake and in intraerythrocytic sodium concentrations. The time course of these changes is illustrated in the case of an individual patient in Figure 1. During the first few days both [^{3}H]-digoxin binding and ^{86}Rb uptake by the red cells fell and thereafter started to fluctuate in parallel with each other. Intraerythrocytic sodium concentrations rose to a new value and did not fluctuate. The QS_2I fell during the first few days and remained constant thereafter. During this time the plasma digoxin concentrations varied between 0.5 and 2.5 ng/ml. It should be noted that the maximum changes in the other measurements occurred when the plasma digoxin concentrations were below 1.0 ng/ml.

Effects on erythrocytic [^{3}H]-digoxin binding, ^{86}Rb uptake and intraerythrocytic sodium concentrations. In Figure 2 the pretreatment values of these red cell measurements are shown together with the corresponding values which occurred in each patient during treatment and before the onset of fluctuations. Erythrocytic [^{3}H]-digoxin binding fell in twelve of seventeen patients studied and ^{86}Rb uptake in twenty-two of thirty-one. The most consistent change after digoxin therapy was a rise in intraerythrocytic sodium concentrations. This failed to occur in only two of the twenty-one patients studied. The distribution of these changes between patients in sinus rhythm and atrial dysrhythmias is given in Table 2. Intracellular potassium concentrations showed no consistent change after digoxin therapy.

Effects on systolic time intervals. The most consistent and pronounced change in the systolic time intervals after digoxin administration was a shortening of the QS_2I (Table 2). In Figure 3 are illustrated the pretreatment values of QS_2I, LVETI and the ratio PEP/LVET for each patient in whom measurements were made and the corresponding minimum values which occurred during digoxin therapy. The mean fall in QS_2I was 40 ms ($P < 0.001$). The LVETI also shortened (mean fall 32 ms, $P < 0.001$). There was no consistent change in either PEP/LVET (0.449 ± 0.156 before treatment and 0.462 ± 0.160 after) or in PEPI (140 ± 22 ms before treatment and 139 ± 23 ms after).

Effects in atrial dysrhythmias. Seven patients were in atrial fibrillation; after digoxin administration two converted to sinus rhythm and five showed a reduction in ventricular rate (mean fall 52 ± 25 beats/min). Two patients were in atrial flutter with block. One who had an initial ventricular rate of 137 beats/min responded to digoxin with a fall in ventricular rate of 41 beats/min. The other had an initial ventricular rate of 95/min (and therefore a high degree of A-V block) and there was no change in

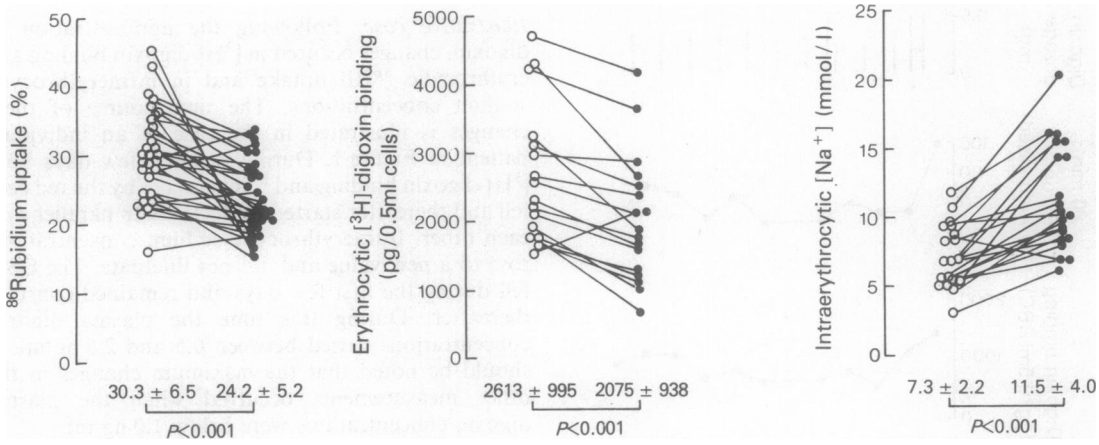

Figure 2 Changes in ^{86}Rb uptake, erythrocytic [^{3}H]-digoxin binding and intra-erythrocytic sodium concentrations. Values on the left (○) are those prior to digoxin administration and values on the right (●) are those during therapy but before the onset of fluctuations. The values during therapy are those corresponding to that day on which maximum changes in red cell measurements and systolic time intervals coincided.

ventricular rate following digoxin administration. These changes are summarized in Table 2.

Concordance of red cell and clinical changes. The numbers of patients in whom individual red cell changes concurred with changes either in QS_2I (patients in sinus rhythm) or in cardiac rate or rhythm (patients with atrial dysrhythmias) are given in Table 3. It can be seen that changes in intraerythrocytic sodium concentration most frequently reflected changes in the clinical variables.

Relationships among [^{3}H]-digoxin binding, ^{86}rubidium uptake, intraerythrocytic sodium concentrations and systolic time intervals. These relationships are summarized in Table 4 and some are illustrated in Figures 3–10. With the exception of the comparison between ^{86}Rb uptake and [^{3}H]-digoxin binding all values shown are those which occurred before the onset of fluctuations.

There were statistically significant correlations among the three red cell measurements illustrated in Figures 4, 5 and 6. Multivariate analysis did not improve the correlations. In the case of the relationship between [^{3}H]-digoxin binding and ^{86}Rb uptake there were significant correlations at times both before and during fluctuations (Figure 4). The correlation between values occurring during the time of fluctuations indicates that the time-course of the fluctuations in these two measurements coincided closely in individual patients (see Figure 1).

In the patients in sinus rhythm, QS_2I correlated with all three red cell measurements (Figures 7, 8 and 9). However there was no correlation between QS_2I and the plasma digoxin concentration (Figure 10). In all cases the corresponding correlations with the other systolic time interval indices were less good. There were weak correlations between plasma digoxin concentration and both [^{3}H]-digoxin binding and intraerythrocytic sodium concentration but not ^{86}Rb uptake.

In the patients with atrial dysrhythmias there were insufficient data to analyse the relationships between red cell measurements and cardiac response.

Table 2 Incidence of changes observed after administration of digoxin

	Fall in ^{86}Rb uptake (>20% compared with pretreatment value)	*Fall in [^{3}H]-digoxin binding (>20% compared with pretreatment value)*	*Rise in intraerythrocytic [Na^+] (>20% compared with pretreatment value)*	*Fall in QS_2I (>20 ms)*	*Fall in LVETI (>20 ms)*	*Response in atrial fibrillation or flutter (slowing of ventricular rate or conversion to S.R.)*
Sinus rhythm	16/22	10/14	15/16	16/18	12/15	—
Atrial	6/9	2/3	4/5	—	—	

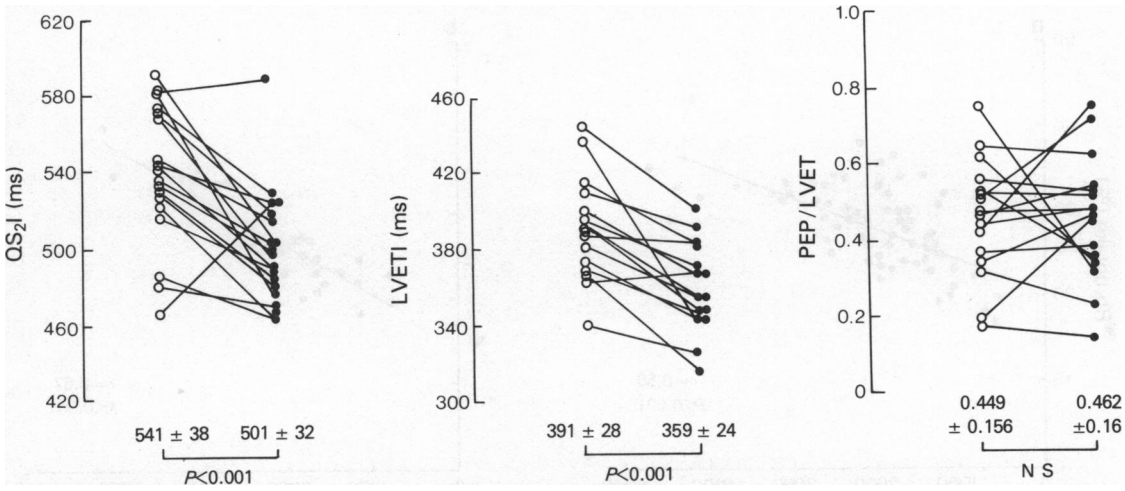

Figure 3 Changes in systolic time intervals following administration of digoxin. The values on the left (○) are those before digoxin therapy, values on the right (●) are those during therapy but before the onset of fluctuations. The values during therapy are those corresponding to that day on which maximum changes in systolic time intervals and red cell measurements coincided.

Discussion

There is considerable evidence that the membrane-bound Na^+,K^+-ATPase is responsible for transport of sodium and potassium across cell membranes (Glynn & Karlish, 1975). Both the enzyme and active cation transport are inhibited *in vitro* by cardiac glycosides and there is evidence to suggest that the mechanism of action of cardiac glycosides (of both electrophysiological and positive inotropic effects) is related to that inhibition (Schwartz, Lindenmayer & Allen, 1975).

^{86}Rb is handled by the red cell membrane in the same way as ^{42}K (Love & Burch, 1953). In a previous study, transport of ^{86}Rb by the erythrocytes of patients receiving digoxin was shown to be inhibited

Table 3 Concordance of red cell and clinical changes*

A. Patients in sinus rhythm

	Red cell change reflects change in QS_2I	*Red cell change does not reflect change in QS_2I*
^{86}Rubidium uptake	11	7
[^{3}H]-Digoxin binding	8	2
Intraerythrocytic [Na^+]	12	0

B. Patients in atrial fibrillation

	Red cell change reflects clinical response	*Red cell change does not reflect clinical response*
^{86}Rubidium uptake	5	4
[^{3}H]-Digoxin binding	2	1
Intraerythrocytic [Na^+]	4	1

*Defined as in Table 2.

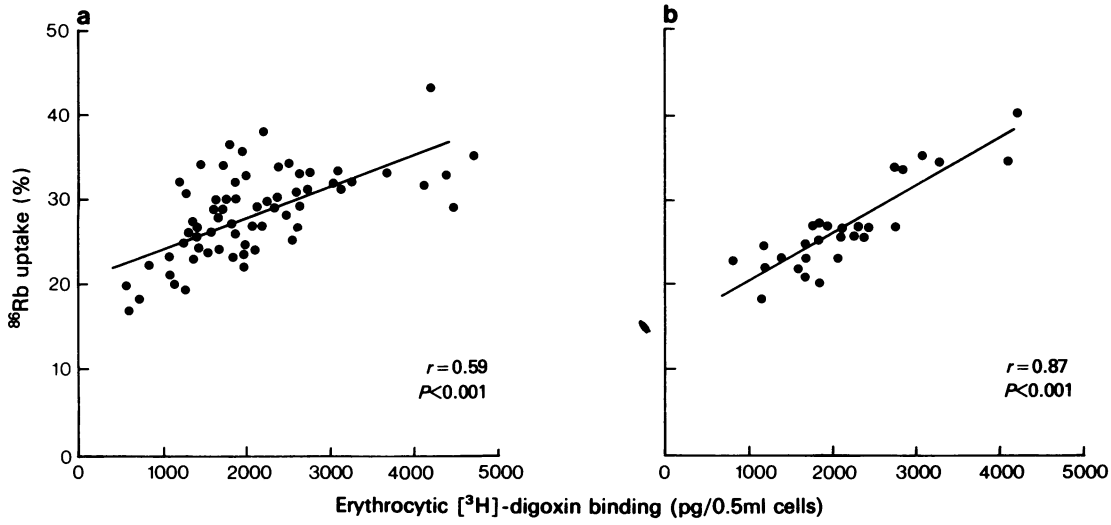

Figure 4 The relationship between ^{86}Rb uptake and *in vitro* [^{3}H]-digoxin binding. (a) shows values before the onset of fluctuations and (b) those values which occurred during fluctuations.

compared with pre-treatment values. The extent of this inhibition correlated well with the reduction in ventricular rate which occurred after administration of the drug to patients with atrial fibrillation (Aronson *et al.*, 1977).

We have extended this work by studying changes in ^{86}Rb uptake, *in vitro* [^{3}H]-digoxin binding to erythrocytes and intraerythrocytic sodium concentration after administration of digoxin in patients in sinus rhythm or in atrial fibrillation or flutter.

Changes in [^{3}H]-digoxin binding and ^{86}rubidium uptake by erythrocytes and intra-erythrocytic sodium concentration. As was found in the study of patients with atrial dysrhythmias, *in vitro* ^{86}Rb uptake by erythrocytes was inhibited in the majority of patients during the first few days of therapy with digoxin and fluctuations in ^{86}Rb uptake occurred thereafter. We have now also shown that [^{3}H]-digoxin binding falls during the first few days of treatment and then starts to fluctuate, the changes both before and during fluctuations occurring in parallel with the changes in ^{86}Rb uptake (Figures 1 and 4).

This initial reduction in *in vitro* [^{3}H]-digoxin binding is probably due to occupation of the red cell membrane glycoside-binding sites by digoxin already bound *in vivo*. From the characteristics of the binding of digoxin to red cell membranes (Ford, Aronson,

Table 4 Two variable correlations among biochemical variables, systolic time intervals and plasma digoxin concentration (PDC)

Dependent variable	*Independent variable*	*Patients included*	*r*	*P*
^{86}Rb uptake	[^{3}H]-Digoxin binding	SR and AF	0.658*	<0.001
^{86}Rb uptake	Intracellular [Na^+]		−0.561	<0.001
[^{3}H]-Digoxin binding	Intracellular [Na^+]		−0.556	<0.001
QS_2I	^{86}Rb uptake	SR only	0.547	<0.001
QS_2I	[^{3}H]-Digoxin binding	SR only	0.705	<0.001
QS_2I	Intracellular [Na^+]	SR only	0.578	<0.001
QS_2I	PDC	SR only	0.01	n.s.
[^{3}H]-Digoxin binding	PDC	SR and AF	−0.300	<0.01
^{86}Rb uptake	PDC		−0.230	<0.05
Intracellular [Na^+]	PDC		0.09	n.s.

*Correlation for pooled values before and during fluctuations. For separate correlations see Figure 4. SR sinus rhythm; AF atrial fibrillation.

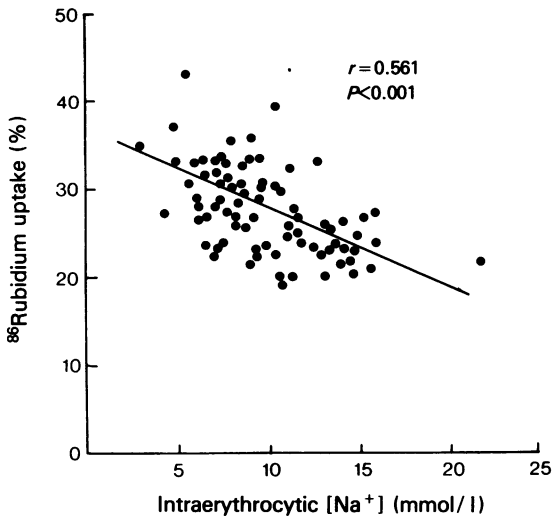

Figure 5 The relationship between ^{86}Rb uptake and intra-erythrocytic sodium concentrations. Values shown are those prior to the onset of fluctuations during acute digoxin therapy.

Grahame-Smith & Rose, 1979) we are confident that during the performance of the binding assay digoxin already bound to the red cell membranes will not be removed by washing the cells, will not be displaced by high concentrations of [^{3}H]-digoxin and will not spontaneously dissociate during the relatively short incubation time. Preincubation *in vitro* of red cells with various concentrations of unlabelled digoxin reduces in stoichiometric fashion the amounts of [^{3}H]-digoxin that can be bound and does not alter the apparent dissociation constant (K_D) for binding of [^{3}H]-digoxin (Ford *et al.*, 1979).

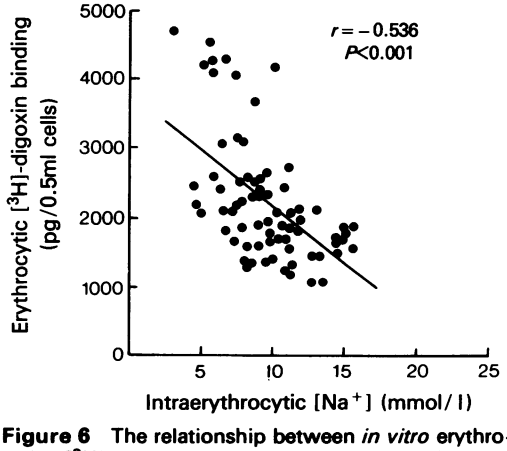

Figure 6 The relationship between *in vitro* erythrocytic [^{3}H]-digoxin binding and intra-erythrocytic sodium concentrations. Values shown are those prior to the onset of fluctuations, during acute digoxin therapy.

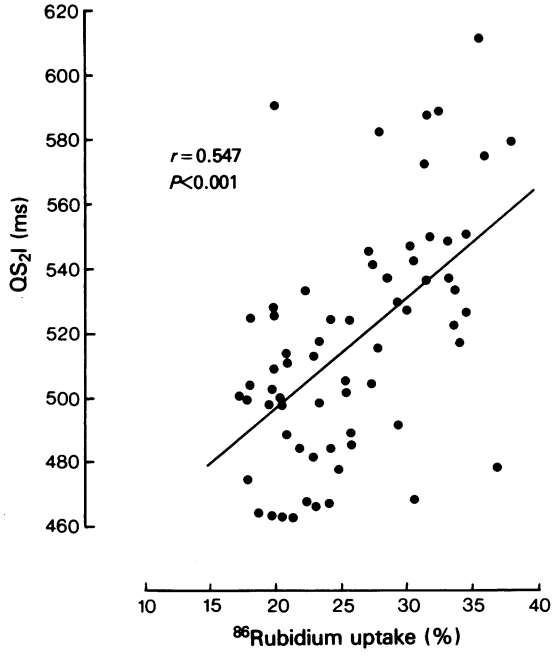

Figure 7 The relationship between QS_2I and ^{86}Rb uptake. Values shown are those prior to the onset of fluctuations.

Thus the *in vitro* binding of [^{3}H]-digoxin should afford an indirect measure of the occupancy of glycoside receptors by unlabelled digoxin already bound *in vivo*. A similar suggestion has been made by Allen, Entman & Schwartz (1975) to explain the reductions in [^{3}H]-ouabain binding to partially purified Na^+,K^+-ATPase from canine heart muscle found after Langendorff perfusion with various concentrations of unlabelled ouabain. The percentage reduction in [^{3}H]-ouabain binding from control values was proportional to the concentration of unlabelled ouabain used in the perfusion and was also closely related to the percentage inhibition of Na^+,K^+-ATPase activity after perfusion with the same concentration.

It has previously been reported (Kettlewell, Nowers & White, 1972; Astrup, 1974; Funder & Wieth, 1974) that a rise in intra-erythrocytic sodium concentration occurs in patients after administration of cardiac glycosides. A steady state was only reached after several days even when the glycoside was given intravenously (Funder & Wieth, 1974). We have confirmed that an increase in intra-erythrocytic sodium concentration occurs after administration of digoxin. In contrast to the authors cited above and to Clifford & Beautyman (1958) we did not find any consistent change in intra-erythrocytic potassium concentrations.

The changes in [^{3}H]-digoxin binding, ^{86}Rb uptake

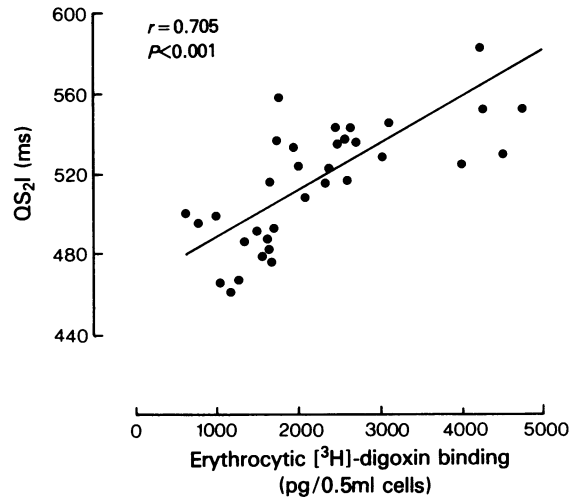

Figure 8 The relationship between QS_2I and *in vitro* [^{3}H]-digoxin binding. Only values which occurred prior to the onset of fluctuations are shown.

and intra-erythrocytic sodium concentration which occur during the first few days of digoxin treatment suggest that there is partial occupation of red cell glycoside receptors *in vivo* with concomitant inhibition of Na^+,K^+-ATPase and consequent elevation of intra-erythrocytic sodium concentration to a new, higher steady-state value.

The fluctuations in [^{3}H]-digoxin binding and ^{86}Rb uptake, occurring as they do in parallel, can be taken to indicate changes in the number of receptor sites available for *in vitro* occupation and cation pumping. Such changes might be due to changes in the amount of digoxin bound to the red cell membrane *in vivo*, to alterations in the total number of binding sites in the red cell membrane or to a combination of the two. We are at present unable to distinguish among these possibilities. One might also expect fluctuations to occur in intra-erythrocytic sodium concentration, fluctuations which would be of sufficient magnitude to be detected by our measurement technique if they were of similar proportion to the fluctuations in the other measurements. At present we have no clear explanation for the absence of fluctuations in intra-erythrocytic sodium concentration at times when [^{3}H]-digoxin binding and ^{86}Rb uptake *are* fluctuating.

Changes in systolic time intervals after digoxin administration

Measurement of the systolic time intervals offers a convenient, easily repeated, non-invasive way of estimating cardiac performance. The most consistent and pronounced change in the systolic time intervals after administration of cardiac glycosides has been reported to be that which occurs in QS_2I although this change correlated poorly with measured haemodynamic changes (Weissler & Schoenfeld, 1970). We have also found that the most consistent and pronounced change after administration of digoxin was that which occurred in QS_2I. Consistent changes of smaller magnitude also occurred in LVETI (Figure 3).

It has previously been shown that after myocardial infarction there is a shortening of LVETI but not of QS_2I over the first four days (Brubakk & Overskeid, 1976). Some of our patients were studied in the period after a myocardial infarction and we therefore analysed the changes in systolic time intervals in these patients and compared them with the changes which occurred in the others. LVETI fell in both types of patients and there was no significant difference in the magnitude of the changes which occurred in the two types. We therefore feel that it is justifiable to attribute the changes in LVETI as well as in QS_2I to digoxin administration.

Relationships among [^{3}H]-digoxin binding, ^{86}Rb uptake, intra-erythrocytic sodium concentrations, plasma digoxin concentrations and systolic time intervals

The relationships among the various red cell measurements, the plasma digoxin concentrations and the QS_2I are listed in Table 4 and illustrated in Figures 3–10. The reduced *in vitro* [^{3}H]-digoxin binding which occurs during digoxin therapy indicates occupation of erythrocyte binding sites by digoxin already bound *in vivo*, and the alterations in cation flux and intracellular sodium concentration represent a pharmacodynamic effect of this digoxin bound to the erythrocyte. Thus the relationships

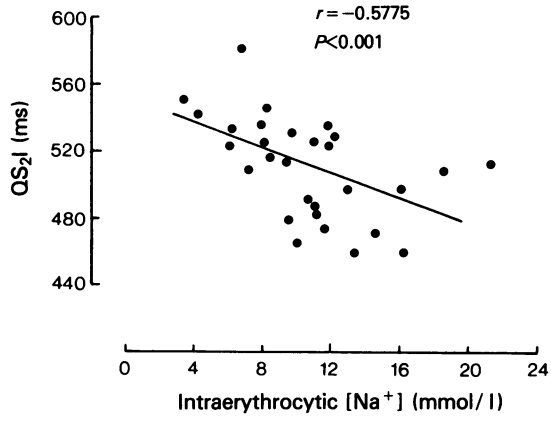

Figure 9 The relationship between QS_2I and intra-erythrocytic sodium concentrations.

found among [^{3}H]-digoxin binding, ^{86}Rb uptake and intra-erythrocytic sodium concentrations (Figures 4–6) are not unexpected.

The demonstration of relationships between these erythrocyte changes and the QS_2I (an index of the cardiac response to digoxin) (Figures 7–9) suggests that certain pharmacodynamic events involving the action of digoxin on membrane Na^+,K^+-ATPase in the erythrocyte mirror those occurring in the heart. This view is supported by the observation of Erdmann & Hasse (1975) that the glycoside receptors of human erythrocytic and cardiac membranes have the same apparent dissociation constant (K_D) for ouabain and thus the same affinity for the drug.

On the other hand, the lack of correlation between QS_2I and plasma digoxin concentrations (Figure 10) simply reflects the uncertainty of the relationship between the concentrations of digoxin in the plasma and those in the heart and our ignorance of what function of the plasma digoxin concentration *v.* time curve determines the amount of digoxin which binds to cardiac receptors. The relationships between plasma digoxin concentrations and the red cell variables are listed in Table 4. We are uncertain of the meaning of these weak correlations. It may simply be that the statistical significance of two of the relationships has occurred by chance but we would not rule out the possibility of a real relationship.

Conclusions

In this study we set out to investigate the relationships among various markers of a pharmacodynamic effect of digoxin on patients' red cells, plasma digoxin concentrations and the cardiac response to digoxin therapy. Our approach has been based on the observation that cardiac glycosides inhibit cation flux in erythrocytes and that glycoside binding sites exist in erythrocytes (Hoffman, 1969).

During the first few days of digoxin therapy there were falls in ^{86}Rb uptake and in *in vitro* [^{3}H]-digoxin

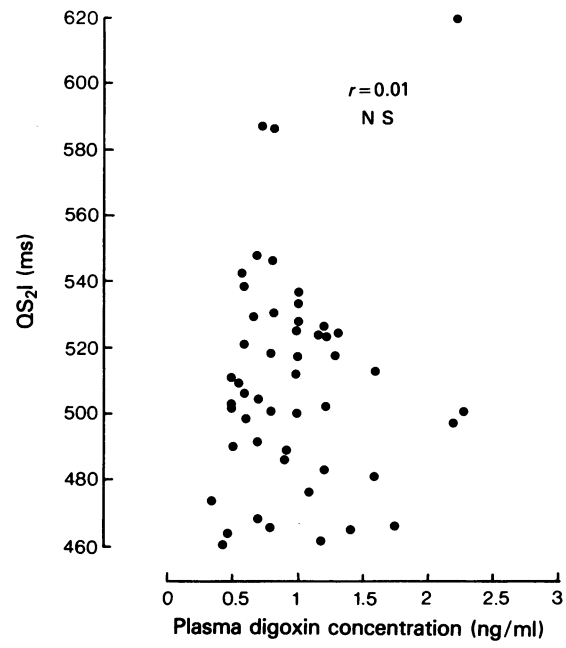

Figure 10 The relationship between QS_2I and plasma digoxin concentrations.

binding and an increase in intra-erythrocytic sodium concentration. Thereafter fluctuations in ^{86}Rb uptake occurred and these fluctuations were accompanied by coincident fluctuations in *in vitro* [^{3}H]-digoxin binding but not in intra-erythrocytic sodium concentrations nor in QS_2I.

In the majority of cases there was concordance between the occurrence of both red cell changes and the cardiac response. The intra-erythrocytic sodium concentration was most frequently concordant (Table 3). Furthermore there were significant correlations between the red cell measurements and the QS_2I in patients in sinus rhythm. There was no such correlation between QS_2I and plasma digoxin concentrations. In view of the fact that the affinity of digitalis for glycoside receptors is the same in the membranes of human cardiac muscle and erythrocytes (Erdmann & Hasse, 1975) the data we have presented provide further evidence that the red cell might be a peripheral marker of central events occurring in the heart. In contrast we have not found plasma digoxin concentrations to be useful in this respect. Although the QS_2I, of all the systolic time intervals, provides the best index of the effect of digitalis on the heart, it does not correlate well with measured haemodynamic changes (Weissler *et al.*, 1970). In this study, however, we have been primarily concerned with determining whether changes in the red cell measurements reflect effects of digoxin on the heart and not with the assessment of the functional haemodynamic changes occurring after digoxin administration.

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