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MONITORING DIGOXIN THERAPY: I. PLASMA CONCENTRATIONS AND AN *in vitro* ASSAY OF TISSUE RESPONSE

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1 An *in vitro* technique is described for measuring the uptake of ^{86}Rb by human erythrocytes.

2 Fifteen patients were treated with digoxin for atrial fibrillation and other fast arrhythmias.

3 ^{86}Rb uptake by the patients' own red cells fell from pre-treatment values during digitalization.

4 The therapeutic response of patients with atrial fibrillation correlated better with the changes in ^{86}Rb uptake than with plasma digoxin concentrations.

Introduction

Measurements of plasma (or serum) glycoside concentrations have been widely used as an aid in the diagnosis of digitalis toxicity and numerous studies have been carried out in an attempt to assess their clinical value. These studies have been critically reviewed recently by Ingelfinger & Goldman (1976) and they have concluded that the usefulness of plasma glycoside concentration measurements in this context has not yet been properly defined. One difficulty lies in the considerable overlap between 'therapeutic' and 'toxic' concentrations and indeed it is evident that in many studies there is a wide range of concentrations apparently compatible with a 'therapeutic' status in individual patients.

The overall problem of monitoring digoxin therapy by measuring plasma digoxin concentrations at a given time after a dose is a complex one because little is known about the precise relationships among the factors involved in the following sequence of events determining the therapeutic outcome:

1. The time course of plasma concentrations after doses of digoxin.
2. Concentrations and binding of digoxin at specific cardiac sites.
3. The biochemical and pharmacological effects.
4. The therapeutic effect.

Undoubtedly there are variables, such as alterations in electrolyte balance and in renal and thyroid function, which are involved in the quantitative linkage of these four steps and which are therefore responsible for difficulties in interpretation of plasma glycoside concentrations.

The aim of these studies has been to develop and explore a technique which would give an indication of a pharmacological effect of digoxin which might possibly reflect a pharmacodynamic effect of digoxin on the heart, to relate this pharmacological effect to plasma digoxin concentration measurements and to see if this pharmacological effect bears any relationship to the therapeutic effect of digoxin.

The rationale of the technique depends on the inhibition of the enzyme sodium- and potassium-linked magnesium-dependent adenosine triphosphatase ($Na^+-K^+-ATPase$) by cardiac glycosides. There is evidence to suggest that inhibition of this enzyme is the mechanism of action of cardiac glycosides, causing their electrophysiological effects and possibly mediating their positive inotropic effects (Schwartz, Lindenmayer & Allen, 1975), although it has also been suggested that stimulation of the enzyme may be of importance with regard to the positive inotropic effects (Blood, 1975; Cohen, Daut & Noble, 1975). Inhibition of $Na^+-K^+-ATPase$ in erythrocytes *in vitro* is accompanied by a diminished ability of the erythrocytic membranes to transport potassium into the cell (Schatzmann, 1953). The radioactive isotope of potassium, ^{42}K, has a short half-time of decay and ^{86}rubidium (^{86}Rb), whose half-time of decay is longer (Wang & Willis, 1965), is handled in the same way as ^{42}K by the red cell membrane

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Table 1 Clinical details of the fifteen patients studied

Group	Patient number	Age (years) and sex	Arrhythmia and cause	Digoxin regimen (mg)	Other treatment during period of this study*	Na^+ (mmol/l)	K^+ (mmol/l)	HCO_3^+ (mmol/l)	Urea (mmol/l)	Creatinine (μmol/l)
I	1	80, F	AF, IHD	0.75 over 16 h then 0.25 daily	None	136	4.2	26	4.83	86.6
	2	51, M	AF, RHD	2.0 over 16 h then 0.25 daily	Warfarin	142	4.2	29	4.50	115.8
	3	87, F	AF, IHD	0.5 over 18 h then 0.25 daily	None	125	3.5	29	3.66	74.2
	4	63, F	AF, RHD	1.25 over 6 h then 0.25 daily	Bendrofluazide, potassium, neomycin	137	3.7	23	4.33	76.9
	5	M	AF, IHD		Not known	—	—	—	—	—
	6	59, M	AF, IHD, CCF	0.5 once then 0.25 daily. No response so 1.125 over 28 h then 0.5 daily	Frusemide, potassium, warfarin, diazepam	140	4.1	26	8.33	117.5
	7	64, M	AF, AMI	2.75 over 48 h then stopped because of lack of response	Streptomycin, isoniazid, thiacetazone, cotrimoxazole	142	3.2	28	7.16	130.5
Ia	8	59, F	AF, IHD	2.5 over 48 h then 0.625 (average) daily	Bendrofluazide, frusemide, potassium, theophylline	143	3.7	—	—	—
	9	67, F	AF, IHD	0.75 over 6 h then 0.25 daily for 3 days then 0.5 daily	Frusemide, potassium insulin, heparin, nitrazepam, cotrimoxazole	136	4.1	27	6.99	96.3
	10	35, M	AF, lone fibrillation	1.5 over 19 h then 0.5 daily	None	143	3.8	—	5.83	91.9
II	11	50, F	SVES, IHD, CCF	1.0 over 10 h then 0.25 daily	Bendrofluazide (5 days) then frusemide, potassium	138	3.8	30	3.83	81.3
	12	60, M	SVT, AMI	1.0 over 10 h then 0.25 daily	Frusemide (dose halved after 7 days) potassium, diazepam	139	3.8	30	6.16	91.9
	13	42, M	SVT, AMI	1.0 over 9 h then 0.25 daily	Diazepam, nitrazepam, aspirin, dipyridamole	133	4.7	27	5.49	89.3
	14	73, M	SVT + 2 : 1 block, AMI	0.75 over 28 h then 0.25 daily	Frusemide, potassium, ampicillin, prednisolone	140	4.0	27	6.16	90.1
	15	50, F	SVT, IHD, CCF	0.75 over 10 h then 0.25 daily	Bendrofluazide, potassium, dichloralphenazone, metopirone, o-PPDD	137	3.1	30	5.33	85.7
						(135-145)	(3.5-5.0)	(24-30)	(2.5-6.7)	(70-150)

AMI: acute myocardial ischaemia;
AF: atrial fibrillation;
IHD: ischaemic heart disease (chronic);
RHD: rheumatic heart disease;
SVES: supraventricular extrasystoles;
SVT: supraventricular tachycardia;
CCF: congestive cardiac failure (with peripheral oedema);

Values in brackets are the normal ranges

* Potassium was always administered as the chloride salt

(Love & Burch, 1953; Bernstein & Israel, 1970). We have therefore studied the ability of patients' own red cells to accumulate ^{86}Rb both before and during therapy with digoxin and compared it with the patients' plasma digoxin concentrations and with their clinical response to the drug. Because of the need for a simple and precise means of monitoring clinical progress in the initial stages of assessing this technique we have chosen to study patients with atrial fibrillation and other supraventricular tachyarrhythmias. Preliminary reports of this work have already been published elsewhere (Hibble & Grahame-Smith, 1972; Aronson, Arthur, Grahame-Smith & Hallis, 1975).

Methods

Radioactive rubidium (^{86}RbCl, specific activity 4 mCi/mg) was dissolved in 154 mmol/l NaCl to give a Rb concentration of 12 μmol/l. A potassium-free Ringer solution was made as follows: 154 mmol/l NaCl, 11; 110 mmol/l $CaCl_2$, 15 ml; 155 mmol/l $MgSO_4.7H_2O$, 10 ml; 100 mmol/l phosphate buffer, pH 7.4 (110 mmol/l $Na_2HPO_4.2H_2O$ in 0.02N HCl), 210 ml; glucose 1 g/l. Final concentrations (mmol/l) were: sodium 143.4; calcium, 1.34; magnesium, 1.26; chloride, 126.0; glucose, 5.56.

Patients' venous blood was collected into lithium heparin tubes, centrifuged and the plasma separated. The buffy coat was discarded and the red cells washed three times in 154 mmol/l NaCl by alternate centrifugation and resuspension at 4°C; the concentration of potassium in the final washings was less than 0.04 mmol/l.

Washed packed red cells (1 ml) were mixed with Ringer solution (1.5 ml) and ^{86}RbCl solution (0.5 ml) (final extracellular concentration 3 μmol/l) in a 25 ml Erlenmeyer flask and aliquots (0.1 ml) taken into 50 x 9.5 mm glass test-tubes. One pair of aliquots was left at room temperature while another pair was incubated at 40°C for 60 min following which the red cells were washed three times in 154 mmol/l NaCl at 4°C. Radioactivity present in the final washings registered no greater than background. The aliquots left at room temperature were not washed. Radioactivity in the samples was counted using a Wallac 80,000 gamma counter and the amount of activity in the washed red cells was expressed as a percentage of that in the unwashed red cells (60 min ^{86}Rb uptake). To ascertain linearity of uptake of ^{86}Rb during the first 60 min of incubation aliquots were also taken at 0.5 min and at 30 min. The inter-assay coefficient of variation of ^{86}Rb uptake (daily measurements in the same normal volunteer for 3 weeks) was 4.2% (mean uptake 31.8%) and the intra-assay coefficient of variation (ten measurements in the same sample of red cells) was 3.4% (mean uptake 30.6%).

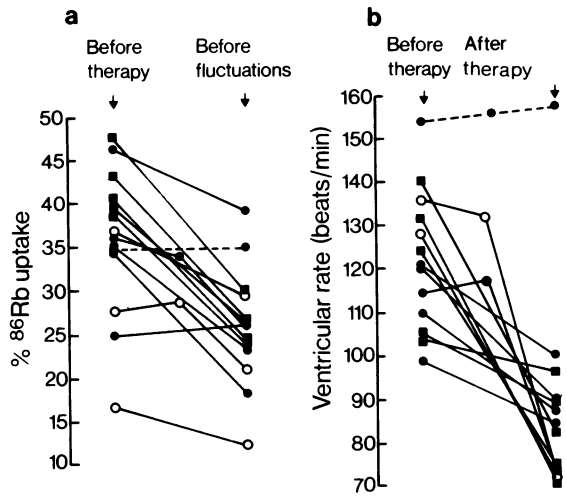

Figure 1 Absolute values of a) ^{86}Rb uptake ($t = 6.151$, $P < 0.001$) and b) ventricular rates ($t = 6.181$, $P < 0.001$) before and after treatment with digoxin but before the onset of fluctuations. (A value of ^{86}Rb uptake of 50% is equivalent to an accumulation of 1.5 μmol/l of red cells/h at the concentrations used in these experiments.) The intermediate values in the two patients who failed to respond initially but responded to increased doses of drug are shown and the dotted line joins the points relevant to the patient who responded to DC cardioversion. The key (● ○ ■) corresponds with Groups I, Ia and II respectively. See text for further discussion.

Plasma digoxin concentrations were measured by radioimmunoassay using $12-\alpha-[^3H]$-digoxin (Smith, Butler & Haber, 1969).

Statistical calculations were performed as described in Snedecor & Cochran (1967).

The clinical data of the patients studied are presented in Table 1. None of the patients was known to have thyroid disease. Blood samples were taken before therapy was commenced and at least 6 h after most subsequent doses of digoxin. In all cases blood samples were taken at the same time of day except for the first two or three samples.

Results

Effect of digoxin therapy on patients' erythrocyte ^{86}Rb uptake

In thirteen of the fifteen patients studied erythrocyte ^{86}Rb uptake by the patient's own red cells

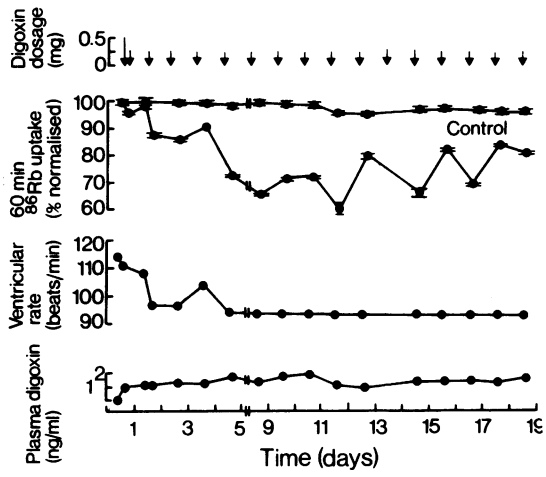

Figure 2 Progress of patient number 1 during the first days of treatment with digoxin (see text for discussion). ^{86}Rb uptake values are shown as the normalized mean and range of duplicates.

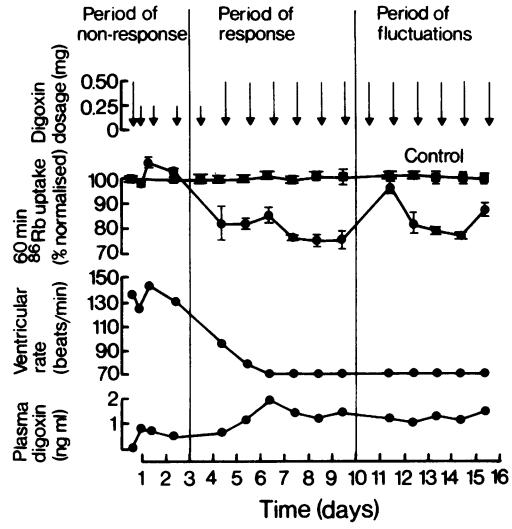

Figure 3 Progress of patient number 9 during the first sixteen days of treatment with digoxin (see text for discussion). ^{86}Rb uptake values are shown as the normalized mean and range of duplicates.

fell when digoxin was given in a dosage sufficient to produce a therapeutic response. These changes in ^{86}Rb uptake are illustrated in Figure 1 alongside the concomitant changes in ventricular rate. The changes are significantly different for both variables ($P < 0.001$).

The time-course of the effect of digoxin therapy on erythrocytic ^{86}Rb uptake is of interest and is illustrated in a case of atrial fibrillation in Figure 2. The fall in ^{86}Rb uptake is generally gradual at the same time as the therapeutic effect and usually reaches a nadir within 5 days. However, within 3 to 11 days ^{86}Rb uptake begins to fluctuate. Both the fall in ^{86}Rb uptake and the subsequent fluctuations have been demonstrated in two normal volunteers who took digoxin. The values shown in Figure 1 for ^{86}Rb uptake after treatment are those which occurred before the onset of fluctuations.

Of the patients whose ^{86}Rb uptake fell during initial digoxin administration two are of particular interest and the progress of one (Case 9) is illustrated in Figure 3. During the first few days of therapy there was neither a change in ^{86}Rb uptake nor a therapeutic response. When digoxin dosage was increased, however, ^{86}Rb uptake fell at the same time as the clinical response occurred. Later on fluctuations commenced. The intermediate values of ^{86}Rb uptake and ventricular rate after therapy had started but before increasing the dose in these two patients are also shown in Figure 1.

Of the two patients whose ^{86}Rb uptakes did not fall one was similar to the two just discussed in that there was also no clinical response; treatment was stopped and sinus rhythm achieved with DC cardioversion of 500 Joules. The remaining patient responded to therapy but there was no change in ^{86}Rb uptake of her red cells.

Relationships among plasma concentrations, ^{86}Rb uptake and the therapeutic response

For the purpose of analysis the patients have been divided into three groups:

Group I: those in atrial fibrillation throughout.

Group Ia: those in atrial fibrillation at the start but who reverted to sinus rhythm during treatment.

Group II: those with other supraventricular tachyarrhythmias.

Figure 4 illustrates the relationship between the percentage change in individual values of ^{86}Rb uptake from the pre-treatment values and the percentage change in ventricular rates at corresponding times. Only values obtained before the onset of fluctuations and, in the case of patients in Group Ia, before reversion to sinus rhythm have been used in the calculations. The correlations between the changes in ventricular rate and changes in ^{86}Rb uptake for the different groups are listed in Table 2. All correlations in Table 2 have been expressed at $100\,r^2$, the percentage

Table 2 List of regression coefficients and degree of significance of the correlations among changes in ventricular rate and ^{86}Rb uptake and plasma digoxin concentrations

Dependent variable	*Independent variables (s)*	*Group (see text)*	*% variation of dependent variable accounted for by regression ($100r^2$)*	*Regression equation (for multiple regression)*	*Level of significance*
ΔV	ΔRb	I	48.47		$P < 0.001$
ΔV	ΔRb	Ia	80.23		$P < 0.001$
ΔV	ΔRb	II	16.42		NS
ΔV	P	I	35.13		$P < 0.005$
ΔV	P	Ia	61.21		$P < 0.005$
ΔV	P	II	11.12		NS
ΔRb	P	I	26.63		$P < 0.01$
ΔRb	P	Ia	40.34		$P < 0.05$
ΔRb	P	II	7.96		NS
ΔV	ΔRb, P	I	56.06	$\Delta V = -0.95 + 0.38\,\Delta Rb + 6.98P$	$P < 0.001$*
ΔV	ΔRb, P	Ia	87.65	$\Delta V = -1.58 + 1.17\,\Delta Rb + 15.76P$	$P < 0.001$*
ΔV	ΔRb, P	II	38.25	$\Delta V = 23.58 + 0.45\,\Delta Rb - 10.44P$	$P = 0.01$*

NS: Not significantly different from zero
ΔV: Percentage change in ventricular rate from pre-treatment
ΔRb: Percentage change in ^{86}Rb uptake from pre-treatment
P: Plasma digoxin concentration (ng/ml)

* In each case the coefficients of both independent variables also differ significantly from zero.

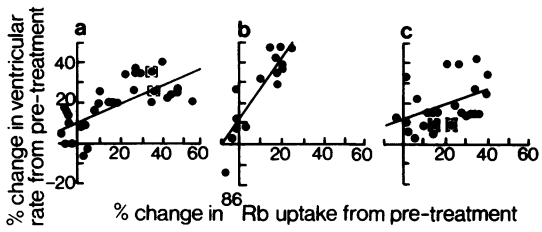

Figure 4 Correlations between the changes in ventricular rate and in ^{86}Rb uptake from pre-treatment values in the three groups of patients during digitalization (a Group I (AF throughout), $r = 0.6962$, $P < 0.001$; b Group IA (AF → SR), $r = 0.8957$, $P < 0.001$; c Group II (other arrhythmias), $r = 0.4052$, NS). The bracketed points, values which occurred when the plasma digoxin concentrations exceeded 3 ng/ml, have been excluded from the analysis but their inclusion does not significantly alter the regression lines.

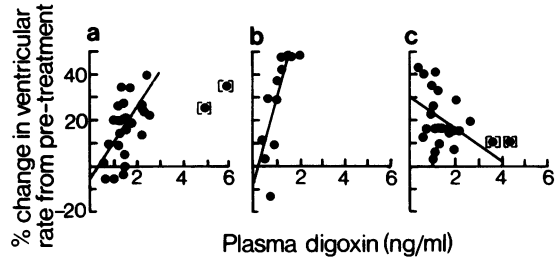

Figure 5 Correlations between the changes in ventricular rate from pre-treatment and plasma digoxin concentrations in the three groups of patients during digitalization (a Group I (AF throughout), $r = 0.5927$, $P < 0.005$; b Group Ia (AF → SR), $r = 0.7824$, $P < 0.005$; c Group II (other arrhythmias), $r = 0.3335$, NS). The bracketed points correspond with those in Figures 4 and 6 and have been excluded from the analysis.

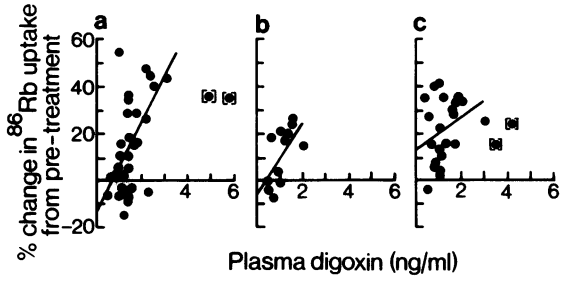

Figure 6 Correlations between the changes in ^{86}Rb uptake from pre-treatment and plasma digoxin concentrations in the three groups of patients during digitalization (a Group I (AF throughout), $r = 0.516$, $P < 0.01$; b Group IA (AF → SR), $r = 0.6351$, $P < 0.05$; c Group II (other arrhyghmias), $r = 0.2821$, NS). The bracketed points correspond with those in Figures 4 and 5 and have been excluded from the analysis.

variation of the dependent variable which is accounted for by the regression.

Figure 5 illustrates the relationships in the three groups between plasma digoxin concentrations and the percentage changes in ventricular rates. The correlations are listed in Table 2.

Figure 6 illustrates the relationships between plasma digoxin concentrations and the percentage changes in ^{86}Rb uptake. The correlations are listed in Table 2.

When multiple regression analysis is applied to all three variables the correlations and regression equations listed in Table 2 are found.

Discussion

In these investigations an attempt has been made to determine to what extent the therapeutic effects of digoxin in atrial fibrillation and other supraventricular tachyarrhythmias may be monitored using a combination of plasma concentration measurement (related to the pharmacokinetic behaviour of the drug) and a test of the drug's pharmacodynamic properties (measured by the ability of the patient's erythrocytes to accumulate ^{86}Rb). Several points of interest emerge from these studies.

Changes in ^{86}Rb uptake during digitalization

During the initial stages of the therapeutic response to digoxin ^{86}Rb uptake by all but two of the patients' erythrocytes fell, this fall being presumably due to inhibition of Na^+-K^+-ATPase although this is not certain Na^+-K^+-ATPase activity not having been measured. Previous studies of the changes which occur in intra-erythrocytic electrolytes during digitalization have shown both an increase in red cell sodium and a decrease in red cell potassium concentrations, changes which are also consistent with inhibition of Na^+-K^+-ATPase in the cell membranes (Clifford & Beautyman, 1958; Kettlewell, Nowers & White, 1972; Funder & Wieth, 1974; Astrup, 1974); whether the alterations in ^{86}Rb uptake occur as a direct consequence of Na^+-K^+-ATPase inhibition or indirectly through changes in intracellular ionic concentrations is not clear.

In three patients with atrial fibrillation there was neither a clinical response nor a fall in ^{86}Rb

uptake despite plasma digoxin concentrations which normally would be accepted as being in the 'therapeutic' range. In two of these three ^{86}Rb uptakes fell and a clinical response occurred when the dose of digoxin was increased. In these two patients the lack of fall of ^{86}Rb uptake with initially ineffective therapy was perhaps an indication of an inadequate pharmacological effect and in this context such knowledge could prove useful, encouraging one to increase the dosage.

Fluctuations

After about 3 to 11 days of treatment ^{86}Rb uptake values consistently began to fluctuate. These fluctuations do not seem to be artefactually related to the ionic composition of the incubating medium—they occur even when only NaCl and glucose are used. Nor are the fluctuations affected by inhibition of that part of the pump which is insensitive to cardiac glycosides by incubation with frusemide (Dunn, 1973) at a concentration of 10 mmol/l for 30 min before measurement of ^{86}Rb uptake. Astrup (1974) reported that the changes he observed in red cell sodium and potassium concentrations were most prominent in high density, older cells, but we have been unable to detect differences in ^{86}Rb uptake in three fractions of cells of different density after centrifugation. Of the studies of changes in intraerythrocytic cationic concentrations quoted above (Funder & Wieth, 1974; Astrup, 1974) which continued for up to thirteen days after starting treatment similar fluctuations did not occur during that period. The fluctuations might therefore be due to secondary changes in Na^+-K^+-ATPase activity necessary to maintain the new cationic equilibrium. Indeed there is evidence that as a consequence of increased intracellular sodium concentration (associated with digitalis therapy, renal failure or hyperthyroidism) there may be secondary *stimulation* of Na^+-K^+-ATPase in human erythrocytes (Funder & Wieth, 1974; Welt, Sachs & McManus, 1964), cardiac muscle (Bluschke, Baum & Green, 1976) and rat kidney (Katz & Lindheimer, 1973). Whether fluctuations in ^{86}Rb uptake are accompanied by similar fluctuations in Na^+-K^+-ATPase activity and intracellular cationic concentrations or not remains to be seen. At present these fluctuations represent a barrier to the wider use of the ^{86}Rb technique as a means of monitoring chronically treated patients.

Correlations of plasma digoxin concentrations, ^{86}Rb uptake values and the clinical response

^{86}Rb uptake values before the onset of fluctuations correlated better with the clinical response than did plasma digoxin concentrations (Table 2). The correlation between changes in ventricular rate and plasma digoxin concentrations illustrated for Group I (patients in atrial fibrillation throughout) in Figure 5 can be compared with that found under similar circumstances by Redfors (1972). In Figure 5a of his paper he plots the absolute ventricular rates in six patients with atrial fibrillation against their plasma digoxin concentrations at different stages of treatment. Calculating from his data the *percentage* changes in ventricular rate form the initial values and plotting those changes against the corresponding plasma digoxin concentrations which he found results in a correlation whose coefficient is 0.6221 ($P < 0.001$) and does not differ significantly from the correlation found in this study (cf. Figure 5, Group I); nor do the slope and intercept on the y-axis of the regression line calculated from his data differ significantly from those depicted in our Figure 5, Group I.

The regression lines of correlation between changes in ventricular rate and changes in ^{86}Rb uptake illustrated in Figure 4 for Groups I and Ia (those who remained in AF and those who reverted to sinus rhythm respectively) differ significantly from one another as regards to slope ($P < 0.001$) but not with respect to the intercept on the y-axis. This suggests that when reversion to sinus rhythm occurs in patients with atrial fibrillation it does so in association with less inhibition of red cell ^{86}Rb uptake than occurs in patients who do not revert but whose ventricular rate is well controlled. There is also evidence that in patients who revert there is increased sensitivity of Na^+-K^+-ATPase to inhibition by digoxin. This suggestion is supported by the observation that the erythrocytes of two patients in Group Ia taken before treatment commenced required less digoxin added *in vitro* to inhibit ^{86}Rb uptake by 50% than did the pre-treatment erythrocytes of three of the patients of Group I (for details see Aronson & Grahame-Smith, 1977) indicating a probable increased *in vitro* sensitivity to the effects of digoxin of the former patients' tissues; there are too few data, however, to analyse statistically. From the study of plasma concentrations (Figure 5) this hint of increased sensitivity in Group Ia would not have been detected.

The lack of correlation between clinical response and both plasma digoxin concentrations and changes in ^{86}Rb uptake in Group II (patients with supraventricular arrhythmias other than AF) is not surprising. Cardiac glycosides do not cause appreciably gradual slowing of the ventricular response in such arrhythmias and one would thus not expect a correlation with the gradual fall in ^{86}Rb uptake which occurred in these patients.

The ability of the measurement of the ^{86}Rb

uptake process to reflect the clinical response in patients with atrial fibrillation seems to be detectable only when there is at least 10% inhibition from pre-treatment levels, judged from the intercepts on the y-axis in Figure 4 for Groups I and Ia; that is, a 10% slowing in ventricular rate would be required before the present techniques would be sufficiently sensitive to detect any change which had already occurred in ^{86}Rb uptake.

As shown in Table 2 the single variate correlations can be further improved in all cases by carrying out multivariate regression analysis. Thus the combined measurement of plasma digoxin concentration and of ^{86}Rb uptake during digitalization for fast arrhythmias gives a better index of response than either measurement alone.

The correlations between percentage change in ^{86}Rb uptake and plasma digoxin concentrations in Group I and Ia shown in Figure 6 (the regressions of which do not differ significantly from each other in any respect) show that the degree of ^{86}Rb uptake inhibition in red cells is related only in small part to the plasma digoxin concentrations. Numerous studies (for references see Härtel, Kyllönen, Merikallio, Ojala, Manninen & Reissell, 1976) have provided conflicting data on the relationship between plasma and myocardial concentrations of digoxin. Similarly conflicting data emerge from studies attempting to show a relationship between plasma concentrations and the measured therapeutic effects of digoxin (for references see Hoeschen & Cuddy, 1975 and Goldman, Probst, Selzer & Cohn, 1975). The fact that plasma concentrations only reflect in part the pharmacodynamic effects of digoxin on the red cell probably indicates the influence of other variables (e.g. changes in electrolyte balance) acting between the pharmacokinetic phase and the final therapeutic effect and perhaps makes it less surprising that plasma concentrations may correlate poorly with both myocardial concentrations and the final therapeutic effect as shown in the studies quoted above. The lack of correlation in Group II suggests that plasma digoxin concentrations are of little value in predicting the tissue response in patients with supraventricular tachyarrhythmias.

Conclusion

The purpose of this study was to explore the usefulness of the technique of measuring red cell ^{86}Rb uptake in monitoring digoxin therapy and to do so we chose to study patients whose response would be easily assessable (patients with fast arrhythmias). We are at present extending our investigations both in patients with cardiac failure in regular rhythm assessing the clinical response by measurement of the systolic time intervals, decreases in which have been shown to correlate well with the effects of cardiac glycosides on the heart (Weissler & Schoenfeld, 1970), and also in patients with digoxin toxicity.

It should be pointed out that these studies provide no direct evidence linking the mode of action of cardiac glycosides to inhibition of $Na^+-K^+-ATPase$. It is known that digoxin inhibits red cell $Na^+-K^+-ATPase$ in therapeutic concentrations (Schwartz *et al.*, 1975) and the changes we have observed may simply have been concomitant with the therapeutic response and not mechanistically responsible for it.

There are two problems which must be overcome if the ^{86}Rb uptake technique is to prove useful in monitoring patients on chronic digoxin therapy. Firstly the cause of the fluctuations in ^{86}Rb uptake, starting at between 3 and 11 days after initial therapy, must be discovered and a means found whereby such fluctuations may be eliminated if possible. Secondly it is at present impossible to tell from a single measurement of the ^{86}Rb uptake of a patient's red cells what degree of inhibition of the uptake process is present compared with pre-treatment cells. We are at present developing the technique (Curd, Smith, Jaton & Haber, 1971) of removing the effects of digoxin from the red cell *in vitro* using antidigoxin antibody so that this degree of inhibition may be estimated (the washing procedure used in the studies and indeed even more rigorous washing does not remove digoxin which is tightly bound to the red cell membrane (Hibble & Grahame-Smith, 1972).

Because the receptors in patients' erythrocytes do not participate in the therapeutic response to digoxin but nonetheless appear to reflect to some extent the therapeutic effects of the drug we would suggest that the term 'spokesman receptors' be applied to such receptors. The use of platelets in monitoring the therapeutic effects of phenothiazines (Boullin, Woods, Grimes, Grahame-Smith, Wiles, Gelder & Kolakowska, 1975) is another such example and it is likely that in the future more such 'spokesmen' may be found to further improve present techniques of monitoring drug therapy.

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