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THE ACUTE CHANGES SEEN IN CARDIAC GLYCOSIDE RECEPTOR SITES, ^{86}RUBIDIUM UPTAKE AND INTRACELLULAR SODIUM CONCENTRATIONS IN THE ERYTHROCYTES OF PATIENTS DURING THE EARLY PHASES OF DIGOXIN THERAPY ARE NOT FOUND DURING CHRONIC THERAPY: PHARMACOLOGICAL AND THERAPEUTIC IMPLICATIONS FOR CHRONIC DIGOXIN THERAPY

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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 various groups of patients—those who have not received digoxin, those during the early phases of treatment, those during chronic (>2 months) treatment, and those toxic.

2 The values of those measurements in the patients in the early phases of therapy and in the toxic patients differed significantly from those of the untreated patients.

3 However, the values in the chronically treated patients were not different from those of the untreated patients.

4 The results suggest that the biochemical pharmacological effects of digoxin which occur during the early phases of therapy do not persist in the long-term.

5 The possible clinical significance of these observations is discussed.

Introduction

In previous studies we have made *in vitro* measurements of several *in vivo* effects of digoxin on patients' erythrocytes to determine the extent to which pharmacodynamic effects of digoxin reflect the clinical response to treatment in non-toxic patients (Aronson, Grahame-Smith, Hallis, Hibble & Wigley, 1977; Ford, Aronson, Grahame-Smith & Carver, 1979b). The observation that cardiac glycosides inhibit sodium and potassium transport in erythrocytes (Schatzmann, 1953) and the supposition that this inhibition is due to inhibition of Na^+, K^+-ATPase activity consequent upon binding of glycoside to the enzyme have formed the basis of our approach. *In vitro* incubation of human erythrocytes with varying concentrations of cardiac glycosides results in the following concentration-related phenomena:

(a) inhibition of the subsequent capacity of the membranes of intact cells to bind [^{3}H]-digoxin *in vitro* (Ford, Aronson, Grahame-Smith & Rose, 1979a);
(b) inhibition of *in vitro* ^{86}rubidium transport into the cells (Aronson & Grahame-Smith, 1977);
(c) increases in intracellular sodium concentrations (unpublished observations).

These observations led us to think that during treatment with digoxin the *in vivo* effects of the drug on patients' erythrocytes would lead to similar changes in these *in vitro* measurements and we have shown that such changes do occur in patients with both atrial fibrillation and cardiac failure in sinus rhythm (Ford *et al.*, 1979b). During the first few days of treatment there were falls in both *in vitro* [^{3}H]-digoxin binding and ^{86}Rb uptake and increases in intraerythrocytic sodium concentrations. After a few days, fluctuations occurred in both [^{3}H]-digoxin binding and ^{86}Rb uptake. Intraerythrocytic sodium concentrations, having risen, did not fluctuate.

In three patients we have been able to continue our studies for a longer period of time. The following case report illustrates how our observations in these patients prompted the present study.

Illustrative case

The patient was a 68-year-old man with cardiac failure in sinus rhythm. He had had a myocardial infarct 12 months previously. He was treated with digoxin and diuretics and changes occurred in *in vitro* [^{3}H]-digoxin binding, ^{86}Rb uptake, intraerythrocytic

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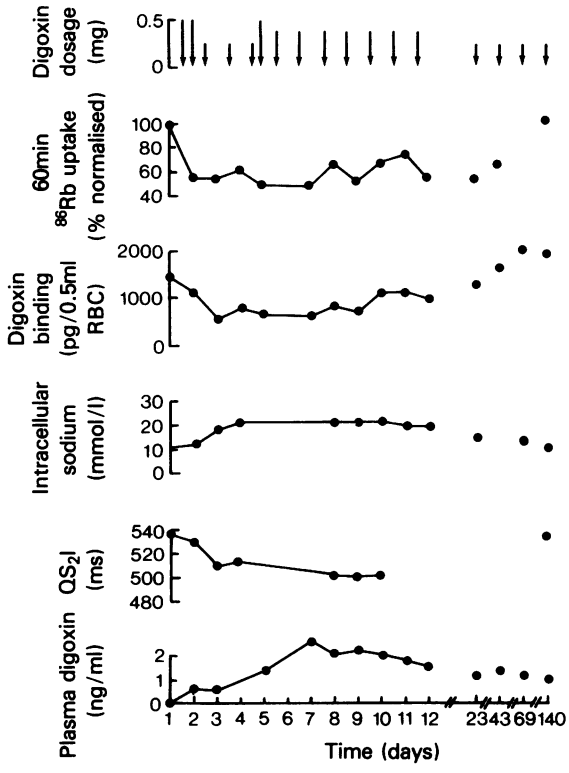

Figure 1 The time course of changes in *in vitro* [^{3}H]-digoxin binding, ^{86}Rb uptake, intraerythrocytic sodium concentrations, QS_2I and plasma digoxin concentrations in the patient whose case is discussed in the text.

sodium concentrations and QS_2I (Figure 1). It should be noted that the maximum changes occurred whilst the plasma digoxin concentrations were less than 1 ng/ml. He improved clinically and after discharge from hospital was seen intermittently over the next 4 months, during which time measurements of [^{3}H]-digoxin binding, ^{86}Rb uptake and intraerythrocytic sodium concentration all returned to pre-treatment values despite plasma digoxin concentrations of about 1 ng/ml. One measurement of QS_2I made 4 months after discharge had also returned to the pre-treatment level. The patient had remained clinically well during this time but suddenly died of a coronary occlusion 4 months after the initial illness.

In two other patients we also observed reversion of the red cell measurements to pre-treatment values after several weeks of treatment. In view of these observations we decided to compare measurement of *in vitro* [^{3}H]-digoxin binding, ^{86}Rb uptake and intraerythrocytic sodium concentrations in patients in the early stages of digoxin therapy with those in patients on chronic therapy. We have also made comparisons with measurements in patients with digoxin toxicity.

Methods

All blood samples were obtained at least 6 h after the most recent dose of digoxin. Plasma digoxin concentrations were measured by radioimmunoassay using 12-α-[^{3}H]-digoxin as radioligand (Smith, Butler & Haber, 1969). The red cells were washed in 112 mM $MgCl_2$ and intraerythrocytic sodium concentrations, erythrocytic [^{3}H]-digoxin binding and ^{86}Rb uptake measured as described previously (Ford *et al.*, 1979b; Aronson *et al.*, 1977).

Statistical comparisons between groups were made using unpaired *t*-tests; where the variances of two groups differed significantly, Cochran's modification of the *t*-test was used (Snedecor & Cochran, 1967).

Patients

The control group consisted of sixty-nine in-patients including thirty-eight who were studied prior to commencing treatment with digoxin and a further thirty-one who did not at any time receive digoxin. Of the thirty-one patients who did not receive digoxin seven were in cardiac failure treated with diuretics and the rest were in hospital for various other reasons.

The acute therapy group consisted of the former thirty-eight patients studied during the early stages of digoxin administration (blood samples were taken at between 2 and 10 days after digitalization).

The chronic therapy group consisted of forty-six patients who had received digoxin for at least 2 months either for the treatment of cardiac failure whilst in sinus rhythm (thirty-one patients) or for atrial dysrhythmias (fifteen patients).

The toxic group consisted of thirteen patients who had digoxin toxicity by criteria previously described (Aronson, Grahame-Smith & Wigley, 1978).

Some of the relevant clinical details of the patients in the various groups are summarized in Table 1. There were no statistical differences among the four groups in terms of age, plasma electrolyte and creatinine concentrations, haemoglobin concentration or red cell indices. There were statistically significantly more men than women in the control and acute groups but not in the chronic and toxic groups.

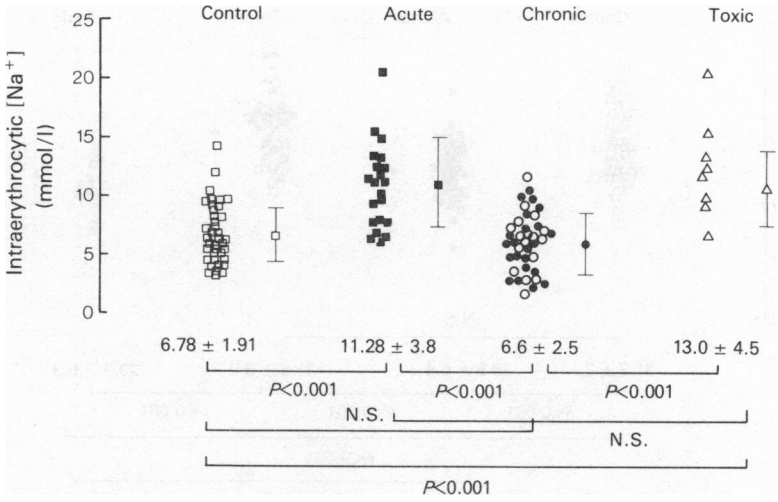

Figure 2 Comparison of intraerythrocytic sodium concentrations in the four groups of patients. In the chronic group the open circles refer to patients with plasma digoxin concentrations less than 0.8 ng/ml.

Results

[^{3}H]-Digoxin binding, ^{86}Rb uptake and intraerythrocytic sodium concentrations in the different groups

The values of these measurements are illustrated in Figures 2, 3 and 4. The mean values of intraerythrocytic sodium concentrations in the control and chronic groups did not differ significantly (Figure 2). The mean values in the acute and toxic groups, which did not differ significantly from one another, were significantly higher than the corresponding means in both the control and chronic groups.

The mean values of ^{86}Rb uptake in the control and chronic groups did not differ significantly from one another (Figure 3); nor did the mean values in the acute and toxic groups differ significantly. The mean values in the acute and toxic groups, however, were significantly lower than the corresponding mean values in both the control and chronic groups. The same relationships occurred in regard to [^{3}H]-digoxin binding with the exception of the comparison between the acute and control groups which did not reach statistical significance (Figure 4). We attribute this lack of difference between these groups to the considerable between-patient variation as there is a statistically significant difference ($P < 0.001$) between the paired values of [^{3}H]-digoxin binding for the seventeen patients in whom measurements of [^{3}H]-digoxin binding were made both before digoxin therapy was begun (control) and after digitalization (acute).

Thus, significant differences were seen between the control and chronic groups on the one hand and the

Table 1 Clinical data of patients

	Control group	*Acute group*	*Chronic group*	*Toxic group*
Number of patients	69	38	46	13
Age (years)	64 (46–90)	63 (47–90)	66 (45–92)	68 (54–93)
Sex M	46	28	29	8
F	23	10	17	5
Diuretics	41	29	37	9
Plasma [Na^+] (mmol/l)	137±4.8	137±3.9	137±4.7	138±8.1
Plasma [K^+] (mmol/l)	4.1±0.46	4.1±0.56	4.3±0.53	3.95±0.72
Plasma creatinine (mmol/l)	128±126	138±148	142±186	163±81
Plasma digoxin (ng/ml)	—	1.2±0.6	1.0±0.5	*3.04±1.4

* Differs from acute and chronic groups $P < 0.001$.

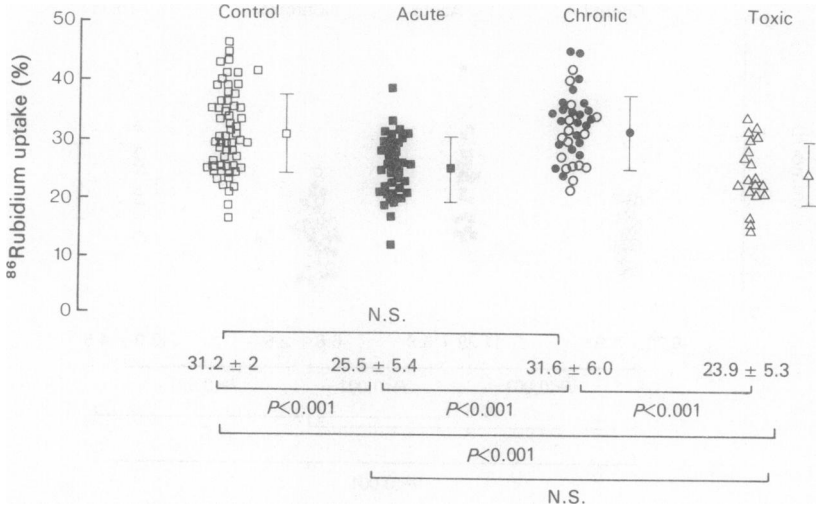

Figure 3 Comparison of ^{86}Rb uptake in the four groups of patients. In the chronic group the open circles refer to patients with plasma digoxin concentrations less than 0.8 ng/ml.

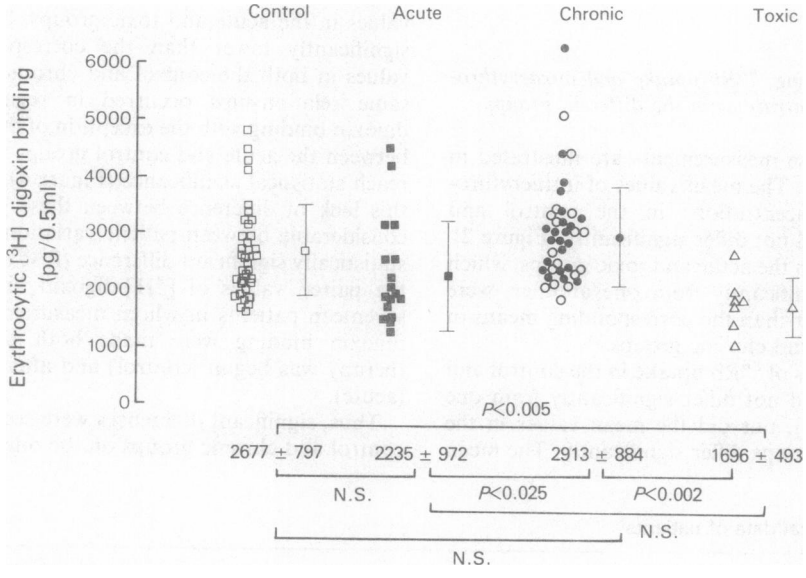

Figure 4 Comparison of [^{3}H]-digoxin binding in the four groups of patients. In the chronic group the open circles refer to patients with plasma digoxin concentrations less than 0.8 ng/ml.

acute and toxic groups on the other, in regard to all three red cell measurements.

The influence of plasma digoxin concentrations on the interpretation of the differences between the groups

There was no significant difference between the mean plasma digoxin concentrations in the acute and chronic groups (means 1.2 and 1.0 ng/ml respectively) but the mean plasma digoxin concentration in the toxic group (3.0 ng/ml) differed significantly from both. Because the mean plasma digoxin concentration in the chronic group was slightly lower than that in the acute group the following analysis was carried out to test whether the difference in the red cell measurements between these two groups was

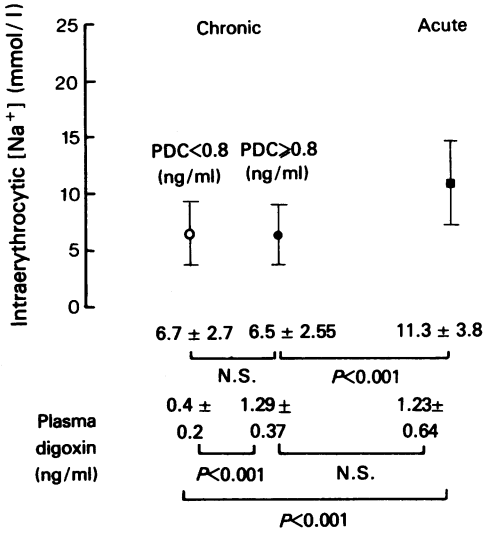

Figure 5 Comparison of intraerythrocytic sodium concentrations in the chronic and acute groups. Effect of plasma digoxin concentration (PDC).

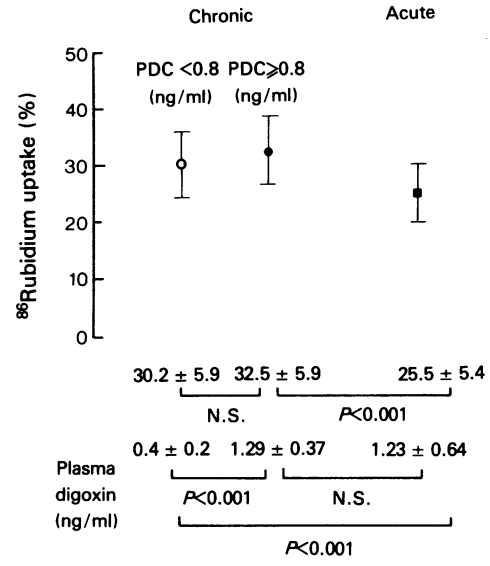

Figure 6 Comparison of ^{86}Rb uptake in the chronic and acute groups. Effect of plasma digoxin concentration (PDC).

attributable to this small difference in plasma digoxin concentration. The patients in the chronic group were divided into those with a plasma digoxin concentration of 0.8 ng/ml or greater and those patients with a plasma concentration of less than 0.8 ng/ml. There was no difference between these sub-groups in regard to ^{86}Rb uptake, [^{3}H]-digoxin binding or intraerythrocytic sodium concentration (Figures 5, 6 and 7). Moreover, when only those patients in the chronic group with a plasma digoxin concentration of greater than 0.8 ng/ml (mean 1.29 ± 0.37) were compared with all those in the acute group (mean plasma digoxin concentration 1.23 ± 0.64) the differences in ^{86}Rb uptake, [^{3}H]-digoxin binding and intraerythrocytic sodium concentrations persisted at the same levels of statistical significance (Figures 5, 6 and 7). Thus the differences in red cell measurements between the patients in the acute and chronic groups cannot be attributed to the small and non-significant difference in plasma digoxin concentration between the two groups.

The effect of other variables

There were differences in the sex distribution in the different groups but when the values of the red cell measurements in the men were compared with those in the women there were no differences within any of the groups. Similarly there were no differences within the groups between patients taking diuretics and those who were not.

Discussion

Red cell changes during acute and chronic digoxin therapy

We have previously shown that during the first few days of administration of digoxin, changes occurred in patients' erythrocytes: ^{86}Rb uptake fell, *in vitro* [^{3}H]-digoxin binding fell and the intraerythrocytic sodium concentration rose (Ford *et al.*, 1979b). These changes correlated with the changes which occurred in QS_2I (total electromechanical systole corrected for heart rate) in patients with cardiac failure in sinus rhythm. Subsequently fluctuations occurred in both ^{86}Rb uptake and *in vitro* [^{3}H]-digoxin binding but not in intraerythrocytic sodium concentrations. In three patients studied for more than one month the red cell measurements gradually returned to pre-treatment values.

In this study we have found no difference in these red cell measurements between untreated patients (control group) and those patients who had been taking digoxin for more than two months (chronic group). This was in contrast to the difference shown between the values in untreated patients and those studied during the first few days of treatment (acute group).

We have interpreted the initial fall in *in vitro* [^{3}H]-digoxin binding as being due to occupation of some of the glycoside-binding sites by digoxin bound *in vivo*, the fall in ^{86}Rb uptake as a consequence of this *in vivo*

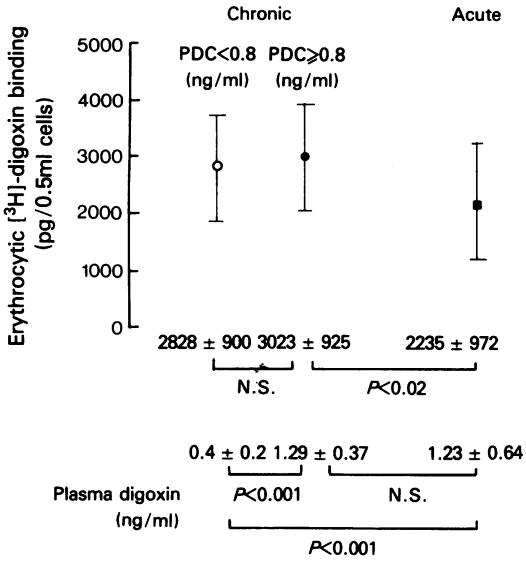

Figure 7 Comparison of [^{3}H]-digoxin binding in the chronic and acute groups. Effect of plasma digoxin concentration (PDC).

occupation and of inhibition of Na^+, K^+-ATPase activity, and the elevated intraerythrocytic sodium concentration as a consequence of this inhibition of cation pumping. The subsequent fluctuations which occur in *in vitro* [^{3}H]-digoxin binding and ^{86}Rb uptake we have interpreted as being a result either of changes in the number of binding sites on the red cell membrane or of alterations in the amount of digoxin bound to the red cell membrane *in vitro* or to a combination of the two.

As an extension of this hypothesis, we would suggest that these changes which we think may be responsible for the fluctuations are also responsible for the eventual restoration of [^{3}H]-digoxin binding, cation pumping (e.g. ^{86}Rb uptake) and intraerythrocytic sodium concentrations to pre-treatment values. That these long-term changes occur despite the continued presence of digoxin in the plasma we have demonstrated above. In order to determine whether there was any change in the amount of digoxin bound to patients' red cell membranes during chronic therapy we measured the amount of digoxin bound *in vivo* to erythrocyte membranes. Digoxin was extracted from washed erythrocyte membranes with dichloromethane. The dichloromethane was evaporated to dryness and the extract reconstituted in horse serum before being measured by radioimmunoassay, the results being expressed in terms of ng/ml of original intact red cells. The mean membrane/plasma concentration ratio was 0.8 ± 0.4 in sixteen patients on chronic treatment and 0.6 ± 0.2 in seven patients within a week of starting treatment (not significantly different). Thus for a given plasma digoxin concentration the amount of digoxin in the red cell membrane was no different in the two types of patient. The differences in [^{3}H]-digoxin binding, ^{86}Rb uptake and intraerythrocytic sodium concentrations between the acute and chronic groups cannot, therefore, be attributed to differences in the amount of digoxin already bound *in vivo* to the red cell membranes of patients in these groups. This makes it likely that the reversion to pre-treatment values, and perhaps the fluctuations, are mediated by changes in the number of sites available on the red cell membrane for binding of digoxin *in vivo*.

The fact that several weeks elapsed before the red cell changes reverted to pre-treatment values in the three patients studied longitudinally suggests that erythrocytes formed during treatment may possess an increased number of available cation-pumping sites as a result of being exposed to digoxin during erythropoiesis. That such adaptive changes may occur following exposure to cardiac glycosides has been demonstrated both *in vitro* and in animals. HeLa cells grown in tissue culture developed an increased number of ouabain-binding sites after prolonged exposure to low concentrations of ouabain (Boardman, Lamb & McCall, 1972; Vaughan & Cook, 1972). This increase in the number of pumping sites was dependent on protein synthesis and resulted in the restoration of cation transport to pre-exposure value (Vaughan & Cook, 1972). The increase was also dependent on *prolonged* elevation of the internal sodium concentration but was not affected by acute changes. A prolonged elevation of the internal sodium concentration with a consequent increase in the number of ouabain-binding sites was also achieved by growing the cells in a medium of low potassium concentration (Boardman *et al.*, 1972). It is also of interest that chronic treatment of guinea pigs with either digitoxin or a potassium-deficient diet resulted in an increase in the Na^+, K^+-ATPase activity of heart muscle (Bluschke, Bonn & Greef, 1976) and that the erythrocytes of patients with chronic hypokalaemia were shown to have increased number of glycoside-binding sites (Erdmann & Krawietz, 1977).

Red cell changes in toxic patients

[^{3}H]-digoxin binding and ^{86}Rb uptake were reduced, and intraerythrocytic sodium concentration was increased in the toxic group of patients compared with the control and chronic groups. There were, however, no significant differences between the means of the comparable values for the acute and toxic groups despite large differences in plasma digoxin concentrations. All of the patients in the toxic group

had been receiving digoxin long term and their red cell measurements would presumably have been comparable, before the onset of toxicity, with those of the patients in the chronic group. When these patients became toxic it would seem that their erythrocytes were then capable of responding to digoxin in the same way as the erythrocytes of patients receiving digoxin for the first time. Before the onset of toxicity such patients would be expected to have values of [^{3}H]-digoxin binding, ^{86}Rb uptake and intracellular sodium concentration similar to patients in the control or chronic groups, and further occupation of binding sites during toxicity might be expected to result in red cell changes of similar magnitude to those which occur during the initial stages of therapy in patients not previously exposed to digoxin.

It is known that the rate of cation pumping and the concentration of cation-pumping sites is much lower in erythrocytes than in other more metabolically active tissues (Baker & Willis, 1972). The magnitude and time-course of changes in digitalis toxicity may well be different in erythrocytes from those changes which might occur in a tissue such as cardiac muscle where there is an increased cation-pumping load because of the repeated depolarizations of the cell membrane. If, however, the erythrocyte changes accurately reflect events in the heart and if there were no difference in cardiac cellular cation pumping between patients in the toxic group and those in the acute group (who are not toxic) then to explain the observed manifestations of toxicity one would have to invoke explanations other than the direct effect on the sodium pump, for example effects mediated by the central nervous system (Gillis, Pearle & Levitt, 1975).

Do the effects of digitalis therapy persist in the long term?

We have shown in this study that the changes which occur in the red cells of patients during the early stages of digoxin therapy do not persist in the long term. We have also shown that these early red cell changes correlate with measurements of QS_2I in patients in sinus rhythm (Ford *et al.*, 1979b). Although changes in QS_2I do not correlate well with measured haemodynamic changes (Weissler & Schoenfeld, 1970) they do represent a cardiac effect of digoxin if not necessarily a therapeutic effect. This raises the question of whether or not the cardiac effects of digoxin persist during chronic therapy.

There are some studies the results of which cast doubt on the efficacy of chronic digoxin administration in patients with cardiac failure in sinus rhythm. Starr & Luchi (1969) were unable to detect any effect after one month of digitoxin administration or of digitoxin withdrawal for 1 month in elderly patients using a ballistocardiographic technique. Davidson & Gibson (1973) studied patients with Starr–Edwards prosthetic aortic values and showed that although there was a shortening of the QA_1 interval 4 to 6 h after digoxin administration, no such effect was detectable after 10 days of continued administration. In a recent study (McHaffie, Purcell, Mitchell-Heggs & Guz, 1978) digoxin therapy over 4 weeks in addition to adequate diuretic therapy, as defined by the achievement of a basal 'dry' body weight, failed to improve the exercise capacity (and the cardiac and respiratory responses to exercise) of six patients with chronic left ventricular failure.

The results of various studies have suggested that maintenance digoxin therapy for patients with heart failure in sinus rhythm can be discontinued without detriment in at least a proportion of patients. Dall (1970) found that digoxin treatment could be stopped in three-quarters of an elderly population in sinus rhythm without clinical deterioration. Many of these patients, however, had had digoxin treatment initiated without unequivocal evidence of the presence of cardiac failure. Hull & Mackintosh (1977) successfully stopped digoxin treatment in seventeen patients receiving digoxin therapy for cardiac failure in sinus rhythm; ten of these patients required an adjustment in other (usually diuretic) therapy but in no case was it thought necessary to reintroduce digoxin. In a study in which treatment was withdrawn from thirty-one elderly patients who had been receiving digoxin for at least a year (Fonrose, Ahlbaum, Bugatch, Cohen, Genovese & Kelly, 1974) fifteen patients did not require further administration of digoxin.

Finally the Liverpool Therapeutic Group (1978) withdrew digitalis therapy from 89 of 159 patients who had previously been in cardiac failure or in atrial fibrillation, all of whom had plasma digoxin concentrations less than 0.8 ng/ml or 'lanatoside C' concentrations less than 0.4 ng/ml. In no case was there recrudescence of cardiac failure or reversal to an abnormal cardiac rhythm. No attempt was made to withhold digoxin from patients with higher plasma digitalis concentrations.

Although these studies suggest that the cardiac effects of digoxin may not persist during chronic treatment there are other studies whose results suggest that withdrawal of digitalis from at least some chronically treated patients in cardiac failure in sinus rhythm results in clinical deterioration (Fonrose *et al.*, 1974—see above; Dobbs, Kenyon & Dobbs, 1977).

There is clearly a need for further study of this problem and if the slow changes in the red cell are of any guide future studies should be carried out over periods of time longer than has hitherto been considered necessary.

References

ARONSON, J.K. & GRAHAME-SMITH, D.G. (1977). Monitoring digoxin therapy. II. Determinants of the apparent volume of distribution. *Br. J. clin. Pharmac.*, **4**, 223–227.

ARONSON, J.K., GRAHAME-SMITH, D.G., HALLIS, K.F., HIBBLE, A. & WIGLEY, F. (1977). Monitoring digoxin therapy. I. Plasma concentrations and an *in vitro* assay of tissue response. *Br. J. clin. Pharmac.*, **4**, 213–221.

ARONSON, J.K., GRAHAME-SMITH, D.G. & WIGLEY, F. (1978). Monitoring digoxin therapy. The use of plasma digoxin concentration measurements in the diagnosis of digoxin toxicity. *Quart. J. Med.*, N.S. **47**, 111–122.

BAKER, P.F. & WILLIS, J.S. (1972). Binding of the cardiac glycoside ouabain to intact cells. *J. Physiol. Lond.*, **224**, 441–462.

BLUSCHKE, V., BONN, R. & GREEF, K. (1976). Increase in the $(Na^+ + K^+)$-ATPase activity in heart muscle after chronic treatment with digoxin or potassium deficient diet. *Eur. J. Pharmac.*, **37**, 189–191.

BOARDMAN, L.J., LAMB, J.F. & McCALL, D. (1972). Uptake of [^{3}H] ouabain and Na pump turnover rates in cells cultured in ouabain. *J. Physiol. Lond.*, **225**, 619–635.

DALL, J.L. (1970). Maintenance digoxin in elderly patients. *Br. med. J.*, **2**, 705–706.

DAVIDSON, C. & GIBSON, D. 1973). Clinical significance of positive inotropic action of digoxin in patients with left ventricular disease. *Br. Heart J.*, **35**, 970–976.

DOBBS, S.M., KENYON, W.I. & DOBBS, R.J. (1977). Maintenance digoxin after an episode of heart failure: placebo-controlled trial in out-patients. *Br. med. J.*, **1**, 749–752.

ERDMANN, E. & KRAWIETZ, W. (1977). Increased number of ouabain binding sites in human erythrocyte membranes in chronic hypokalaemia. *Acta biol. med. germ.*, **36**, 879–882.

FONROSE, H.A., AHLBAUM, N., BUGATCH, E., COHEN, M., GENOVESE, C. & KELLY, J. (1974). The efficacy of digitalis withdrawal in an institutional aged population. *J. Am. ger. Soc.*, **22**, 208–211.

FORD, A.R., ARONSON, J.K., GRAHAME-SMITH, D.G. & ROSE, J.A. (1979a). The characteristics of the binding of 12-α-[^{3}H]-digoxin to the membranes of intact human erythrocytes: relevance to digoxin therapy. *Br. J. clin. Pharmac.*, **8**, 115–124.

FORD, A.R., ARONSON, J.K., GRAHAME-SMITH, D.G. & CARVER, J.G. (1979b). 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. *Br. J. clin. Pharmac.* **8**, 125–134.

GILLIS, R.A., PEARIE, D.L. & LEVITT, B. (1975). Digitalis: A neuroexcitatory drug. *Circulation*, **52**, 739–742.

HULL, S.M. & MACKINTOSH, A. (1977). Discontinuation of maintenance digoxin therapy in General Practice. *Lancet*, **ii**, 1054–1055.

LIVERPOOL THERAPEUTICS GROUP (1978). Use of digitalis in general practice. *Br. med. J.*, **2**, 673–675.

McHAFFIE, D., PURCELL, H., MITCHELL-HEGGS, P. & GUZ, A. (1978). The clinical value of digoxin in patients with heart failure and sinus rhythm. *Quart. J. Med.* (in press).

SCHATZMANN, H.-J. (1953). Herzglycoside als Hemmstoffe für den aktiven Kalium- und Natriumtransport durch die Erythrocytenmembran. *Helv. Physiol. Acta*, **11**, 346–354.

SMITH, T.W., BUTLER, V.P. & HABER, E. (1969). Determination of therapeutic and toxic serum digoxin concentrations by radioimmunoassay. *New Engl. J. Med.*, **281**, 1212–1216.

SNEDECOR, G.W. & COCHRAN, W.G. (1967). *Statistical Methods*, 6th edition. Iowa: Iowa State University Press.

STARR, I. & LUCHI, R.J. (1969). Blind study on the action of digitoxin on elderly women. *Am. Heart J.*, **78**, 740–751.

VAUGHAN, G.L. & COOK, J.S. (1972). Regeneration of cation-transport capacity in HeLa cell membranes after specific blockade by ouabain. *Proc. Nat. Acad. Sci. USA*, **69**, 2627–2631.

WEISSLER, A.M. & SCHOENFELD, C.D. (1970). Effect of digitalis on systolic time intervals in heart failure. *Am. J. med. Sci.*, **259**, 4–20.

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