second paper in this series, which compares neutropenia in UKALL I with that in the second UKALL trial. In UKALL II methotrexate was given in the same overall dosage as UKALL I but in different relationship to mercaptopurine, vincristine, and prednisolone. The schedule in UKALL II, despite essentially the same craniospinal prophylaxis, caused significantly less neutropenia. The same point can be made from the data in this study in that methotrexate is considerably more myelotoxic after previous craniospinal irradiation, even if irradiation is given more than one year before.

The type of fatal infection during remission described in this study is, in general, unlike the serious episodes of infection described by the Memphis group.[4 12 13] They state that "Non-bacterial micro-organisms, particularly *Pneumocystis carinii* and viruses" caused most of their infections. Maintenance treatment in those trials included continuous daily mercaptopurine and weekly methotrexate; C.N.S. prophylaxis was essentially the same as that given in UKALL I. The infections in the second UKALL trial, reported in the next paper in this series, fit more closely the pattern described by the Memphis group. Again, this emphasizes that relatively minor changes in timing of drug administration can result in a totally altered pattern of immunosuppression and myelosuppression and, therefore, drug toxicity while retaining similar antileukaemic activity.

The long-lasting effect of craniospinal irradiation is striking when one recalls that much of the red marrow was not irradiated. The iliac, femoral, and much of the costal marrow will have received only slight scatter exposure. Several points arise from these observations for discussion: how readily can myeloid stem cells move from one part of the body to another? If there is free movement of stem cells why does complete reconstitution not occur from non-irradiated sites? It is possible that repopulation occurs too rapidly. Because irradiation is given in several fractions, stem cells repopulating irradiated marrow after early exposure would be susceptible to damage by subsequent doses of irradiation. This sort of effect is well recognized in relation to recirculating lymphocytes. For instance, Ford[14] has shown that a short-range β-emitting strip placed on a rat spleen can eventually result in gross depletion of the recirculating pool of lymphocytes, as the spleen is a traffic organ for these cells.

Another possible explanation for the longevity of increased susceptibility to methotrexate-induced neutropenia is that irradiation destroys the capacity of bone cavities to support active red marrow. Even if this were so it seems an unlikely explanation for the spleen, liver, and fatty marrow of long bones are known to be reserve sites of myelopoiesis and haemopoiesis. Irradiated marrow could conceivably produce an abscopal effect on distant marrow, as, for instance, a kidney perfused through a stenosed renal artery induces hypertension that damages the contralateral organ. Finally, perhaps the most likely explanation for this long-term effect of irradiation is that maintenance chemotherapy simply prevents or greatly slows myeloid recovery after irradiation damage. In this way a normally short-lived reduction in myeloid capacity could become protracted over many months.

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Serum Digoxin in Patients with Thyroid Disease

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Summary

Serum digoxin concentrations were measured by radioimmunoassay in 17 hyperthyroid and 16 hypothyroid patients after a seven-day course of oral digoxin. The significantly higher levels of serum digoxin in patients with hypothyroidism and lower levels in those with hyperthyroidism were closely related to the measured changes of glomerular filtration rate and digoxin serum half time in these two groups. Differences in serum digoxin concentration contribute to the altered sensitivity to digoxin shown by patients with thyroid disease.

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Introduction

The clinical observation of altered sensitivity to digitalis in patients with thyroid disease has been attributed to a change of intrinsic myocardial function which modifies its response to digitalis, as shown in isolated muscle strip preparations,[1 2] animals,[3] and man.[4] In addition, isotopic studies using single-dose tritiated digoxin[5] and digitoxin or ouabain[6] have shown lower serum levels in hyperthyroidism and higher levels in hypothyroidism. Doherty and Perkins found no significant difference in either digoxin excretion or serum half times and postulated an increased tissue distribution as a possible cause of the low serum levels in hyperthyroid patients.[5]

Our aim was to reassess the influence of renal function on serum digoxin levels in patients with thyroid disease using radioimmunoassay for measurement of serum and urinary digoxin.

Patients and Methods

Studies were performed in 17 hyperthyroid and 16 hypothyroid patients with moderate to severe disease. There were two men in each group. Twenty-four of the 33 studies were performed on hospital

inpatients. All patients gave their informed consent and had normal serum urea and potassium at the start of digoxin treatment. Digoxin (old potency Lanoxin) 0·5 mg was given by mouth as a single daily dose before breakfast for seven days. Serum samples for measurement of digoxin were taken 24 hours after the final dose in all patients. Digoxin serum half times were calculated from samples taken at intervals of eight to 96 hours after the final dose of digoxin when three or more results showed an exponential fall within the limits of assay sensitivity.

A 24-hour creatinine clearance measured on the seventh day was corrected to a surface area of 1·73 m^2. Simultaneous digoxin and creatinine clearances were measured on a two-hour urine sample collected 24 hours after the final dose of digoxin. Serum and urinary creatinine and protein bound iodine (P.B.I.) were measured on a Technicon AutoAnalyzer. The T-3 resin uptake was measured by a modification of the method of Herbert *et al.*[7] The normal range for the free thyroxine index (F.T.I.), derived from the P.B.I. and T-3 resin uptake, was 5-13.

Digoxin Radioimmunoassay.—The assay was a modification of the Lanoxitest kit following the method of Smith *et al.*[8] Serum assay precision, defined by the coefficient of variation for the ranges 0-1·28 and 2·6-10·2 nmol/l (0-1 and 2-8 ng/ml) was 1% and 2·8% respectively. Assay sensitivity was 0·26 nmol/l (0·2 ng/ml). For measurement of urinary digoxin satisfactory results were obtained by using 50 µl of the sample diluted 1/40 with the phosphate buffer. Quality control surveys for the measurement of serum digoxin, run by the American College of Pathologists, gave results for this laboratory within ±0·5 S.D. of the group mean.

Results

As a group hypothyroid patients were both older and heavier (see table). The differences in the F.T.I. reflected the deliberate selection of patients with severe disease.

Mean (±S.D.) Renal Function and Serum Digoxin in Patients with Thyroid Disease. Unpaired Student's t Test was used to assess Significance

	Hyperthyroid Patients	Hyperthyroid Patients	P
No. of patients	17	16	
Age (years)	37 ± 18	54 ± 9	<0·01
Weight (kg)	57·6 ± 9·3	70·9 ± 11·4	<0·001
Free thyroxine index	35 ± 14	3·3 ± 1·3	<0·001
Serum urea (mmol/l)	5·0 ± 1·7	6·3 ± 1·2	<0·05
Serum creatinine (µmol/l)	54·8 ± 12·4	118·5 ± 18·6	<0·001
Corrected creatinine clearance (ml/min)	111·6 ± 34·1	64·4 ± 14·7	<0·001
Serum digoxin (nmol/l)	0·86 ± 0·41	1·87 ± 0·54	<0·001
Serum digoxin t½ (h):			
This study*	30·3 ± 13·6	43·4 ± 11·7	<0·02
Doherty and Perkins[5]†	34·8 ± 5·8	42·7 ± 11·7	N.S.

*Serum digoxin half time measured in 14 hyperthyroid and 14 hypothyroid patients.
†Serum digoxin half time measured in 12 hyperthyroid and 10 hypothyroid patients.
Conversion: SI to Traditional Units—Urea: 1 mmol/l ≈ 6 mg/100 ml. Creatinine: 1 µmol/l ≈ 0·0113 mg/100 ml. Digoxin: 1 nmol/l ≈ 0·78 ng/ml.

GLOMERULAR FILTRATION RATE

There was a highly significant difference in endogenous creatinine clearance with greater depression of the glomerular filtration rate (G.F.R.) than expected from the age difference alone. Thus, only one of the 16 hypothyroid patients had a G.F.R. greater than 80 ml/min, whereas only three of the hyperthyroid patients had a G.F.R. less than 80 ml/min.

Serum creatinine measurements clearly separated the two groups and there was a significant inverse correlation with creatinine clearance (r=0·7). Daily urinary creatinine excretion was significantly reduced in hyperthyroid patients, however, probably because of the relative reduction in lean body mass and creatinine production in this group.[9]

SERUM DIGOXIN

The mean serum digoxin level 24 hours after the final dose (see table) was 0·86 nmol/l (0·67 ng/ml) (range 0·38-2·2 nmol/l (0·3-1·7 ng/ml)) in the hyperthyroid patients and 1·87 nmol/l (1·46 ng/ml) (range 1·28-2·56 nmol/l (1·0-2·0 ng/ml)) in the hypothyroid group. The only hyperthyroid patient with a serum digoxin greater than 1·28 nmol/l (1·0 ng/ml) was a 60-year-old woman in whom digoxin treatment of atrial fibrillation was indicated at the time of admission. After the standard seven-day course she developed overt clinical and electrocardiographic evidence of digitalis toxicity with a ventricular rate of less than 50/min and multiple ventricular ectopics. Serum digoxin nine hours after the seventh dose was 2·8 nmol/l (2·2 ng/ml) and at 24 hours 2·2 nmol/l (1·7 ng/ml). Creatinine clearance at this time was 58 ml/min and was consistently subnormal on repeated measurement despite a normal serum urea and serum creatinine of 70·7 µmol/l (0·8 mg/100 ml).

The digoxin clearance and the creatinine clearance derived from the two-hour urine sample in 25 patients correlated well (r=0·94). The digoxin:creatinine clearance ratio was 1·0±0·2 (±1 S.D.) and not significantly different in the hyperthyroid and hypothyroid patients.

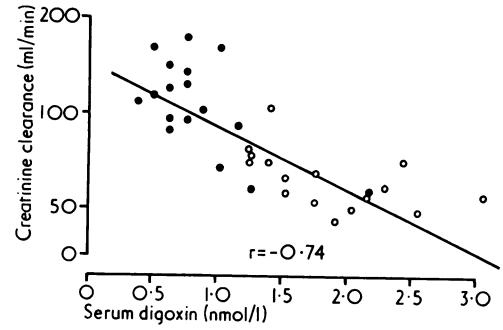

FIG. 1—Relation between 24-hour corrected creatinine clearance and serum digoxin concentration 24 hours after seventh 0·5-mg dose of digoxin in 17 hyperthyroid (●) and 16 hypothyroid (○) patients.
Conversion: SI to Traditional Units—1 nmol/l ≈ 0·78 ng/ml.

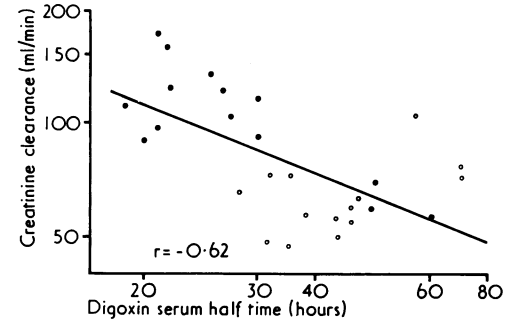

FIG. 2—Relation between creatinine clearance and digoxin serum half time in 14 hyperthyroid (●) and 14 hypothyroid (○) patients.

The relation between serum digoxin 24 hours after the final dose and the 24-hour corrected creatinine clearance is shown in fig. 1. Despite the reduced creatinine clearance of the hypothyroid patients there were no cases of digitalis toxicity in this group. The mean values of digoxin serum half time are shown in the table and compared with the values derived from Doherty and Perkins's study using tritiated digoxin.[5] The relation between digoxin half time and creatinine clearance (fig. 2) further emphasized the continuing dependence of digoxin elimination on renal function despite gross variations in thyroid status. There were, however, some discrepant results in the hypothyroid group. One patient with a normal creatinine clearance of 104 ml/min and a digoxin:creatinine clearance ratio of 1·1 nevertheless had a prolonged digoxin half time of 56 hours. Other hypothyroid patients showed a depressed G.F.R. but normal digoxin half time.

Cumulative urinary digoxin excretion over the seven-day period expressed as a percentage of the given dose was 41% and 39% in two hyperthyroid patients and 23% in one hypothyroid patient.

Discussion

Knowledge of digoxin pharmacokinetics has been derived from studies using tritiated digoxin[10] and from the development and

use of sensitive assays of digoxin in body fluids.[11] This knowledge has recently been reviewed in detail by Smith and Haber.[12] Digoxin in serum is only 23% protein bound and is excreted primarily in the urine in unchanged form or as small amounts of metabolites which are both cardioactive and immunoreactive. Urinary excretion is directly proportional to the glomerular filtration rate, and serum levels in the elimination phase decline exponentially with an average serum half time of 36 hours in patients with normal renal function. Daily maintenance treatment without a loading dose results in a steady-state plateau concentration after four or five half lives or about seven days in patients with normal renal function.[13] Impairment of renal function is associated with higher serum digoxin concentrations at any dose level.

Clearly, therefore, consistent differences in glomerular filtration rate in patients with thyroid disease, of the order observed in our patients, would influence comparative serum digoxin concentrations. In a recent review of renal function in patients with thyroid disease[14] inulin clearance was found to be raised (145·5±5·6 ml/min in 36 hyperthyroid patients reported by seven groups of workers), and in 17 hypothyroid patients G.F.R. fell to 71±4·3 ml/min and then rose towards normal in six out of seven patients who received replacement therapy. In view of the close correlation between digoxin and creatinine clearance the finding of a short digoxin half time and lower serum concentrations of digoxin in the hyperthyroid patients was not unexpected. A similar interpretation is implicit in Doherty's data; mean digoxin clearance was 158 ml/min and 83 ml/min in hyperthyroid and hypothyroid subjects respectively.[5] Undoubtedly a difference in tissue distribution space for digoxin exists in patients with changed thyroid status, but this difference is not relevant to the serum concentrations after achievement of a steady-state plateau in clinical therapeutics.

Serum digoxin concentrations in hyperthyroid subjects could also be low secondary to decreased absorption or increased metabolism or faecal excretion of the drug, factors which might explain our discrepant findings. The demonstration of a relative increase in cumulative urinary digoxin excretion in two hyperthyroid patients despite low serum concentrations argues against an important role for these alternative routes of metabolism or excretion.

A rapid ventricular response to atrial fibrillation refractory to digitalis treatment is common in hyperthyroidism. Chamberlain's finding of a correlation between slowing of the ventricular rate in patients with atrial fibrillation and serum digoxin levels[15] suggested that this apparent refractoriness may be partly due to the lower serum concentration in the hyperthyroid patient. Many clinical studies have established the correlation between serum digoxin levels and therapeutic or toxic effects of the drug, and our findings indicate that ideal digitalization of patients with altered thyroid function requires measurement of both serum digoxin and glomerular filtration rate.

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Isolation and Characterization of an Aetiological Agent in Whipple's Disease

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Summary

A cell wall deficient form of an α-haemolytic streptococcus was grown from a prolonged monolayer cell culture of a lymph node taken from a patient with Whipple's disease. Serological cross reactivity was shown between the organism and the material within Whipple's disease macrophages positive for diastase-resistant periodic acid-Schiff (D./P.A.S.). In vitro studies characterized the organism as a facultative intracellular parasite which caused the accumulation within cells of D./P.A.S.-positive material. These results suggest that a pathogenic bacterium is the essential aetiological agent and that the culture of Whipple's disease tissues in hypertonic media may have practical value.

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Introduction

Whipple's disease is a systemic illness characterized morphologically by the presence of macrophages stained by diastase-resistant periodic acid-Schiff (D./P.A.S.) with maximum involvement occurring in the lamina propria of the proximal small intestine.[1] The aetiology remains an enigma. Indirect evidence strongly suggests the presence of an infectious agent,[2]