A gas chromatographic-mass spectroscopic technique was used to identify dihydrodigitoxin, a metabolite of digitoxin, in the plasma of healthy volunteers and patients with renal failure. Digitoxin and dihydrodigitoxin were extracted from plasma and derivatized with heptafluorobutyric anhydride. In normal subjects, only minimal concentrations of dihydrodigitoxin in plasma could be determined (1 ng/ml) after an intravenous bolus injection of digitoxin. Under a chronic treatment with a daily dose of 0.1 mg digitoxin in three out of seven individuals, detectable dihydrodigitoxin plasma levels were observed (0.7, 1.5, 1.7 ng/ml) (Table 7.1). On the other hand, in seven patients with renal failure, high dihydrodigitoxin plasma concentrations (8.9 ± 0.9 ng/ml) were shown which were in a similar range as those of the parent compound (8.7 ± 2.2 ng/ml) under maintenance treatment with digitoxin.

Pharmacokinetics and metabolism with radioactively labeled cardiac glycosides were examined for the first time in the 1950s and 1960s. In the meantime, methods have become available which also make the determination of concentrations of nonlabeled digitalis glycosides in blood and urine possible. Rubidium uptake method and radioimmunoassay increased our sophistication about the behavior of cardiac glycosides during long-term treatment. Nevertheless, there are many clinical questions which still have to be answered because of the lack of specific assays.

A gas chromatographic method for measuring digoxin and dihydrodigoxin in blood and urine has been designed by Watson and co-workers (Watson et al., 1973). It was shown that dihydrodigoxin is more common than any other degradation product of digoxin. There are no reports yet about the formation of dihydrodigitoxin in the metabolism of digitoxin. Therefore, the present study was designed to investigate the transformation of digitoxin to dihydrodigitoxin in patients using a gas chromatographic-mass spectroscopic technique.
Methods

Patients

Five healthy volunteers, aged 29-37 years, participated in the study. A dose of 1 mg digitoxin (Digimerck®) was injected over a time period of 5 min intravenous. Blood samples were obtained in 24 h intervals for 10 days. Also, in six patients with heart failure who were on a daily oral maintenance dose of digitoxin (Digimerck®), a blood sample was drawn immediately before the next dose was taken. In addition, plasma of seven patients with renal failure (plasma creatinine concentration: Table 7.2) under chronic oral digitoxin treatment (Digimerck®) was analyzed.

Assay

To correct for losses during analysis, 50,000 dpm 3H-digitoxin (15 Ci/mM; New England Nuclear, Frankfurt, Dreieichenhein) were added to 10 ml of plasma prior to extraction and an equal amount was taken as a standard for liquid scintillation counting. The plasma sample was extracted once with six volumes of methylene chloride by stirring for 25 min at room temperature. The methylene chloride was run to a conical glass tube and evaporated under a stream of nitrogen. The residue was redissolved in 3 ml methanol-methylene chloride (1:1) and transferred onto a column (diameter: 1.4 cm; length: 22 cm) packed with florisil (80/100 mesh).

Prior to use, the column was washed with 20 ml of methylene chloride. The effluent was removed. The elution was performed with 20 ml of ethylacetate-methylene chloride-methanol-acetone (1:3:3:4). This solution was evaporated under nitrogen and the residue was transferred using 50 μl methanol-chloroform (1:1) to a silica gel TLC plate (20 x 20 cm; silica gel 60, 0.25 mm layer, Merck, Darmstadt). TLC then was carried out in the solvent system chloroform-acetone (13:7) for three times to a height of 15 cm. Reference substances were chromatographed in separate lanes.

Digitoxin was located by scanning of the thin layer plate in a Berthold scanner (Friezeke a. Hoepfner, mod. LB-2560). The reference compounds were identified using a spray technique described by Kaiser (Kaiser, 1955). The area corresponding to digitoxin was scrapped off the TLC plate and stirred in 10 ml methanol-chloroform (1:1) for 20 min and then centrifuged for 5 min at 5000 xg. The supernatant was evaporated and the residue reconstituted with 2 ml of benzene. The HBF derivative was formed by reacting this solution with 10 μl of heptafluorbutyric anhydride (Pierce Chemical Comp., Rockford, U.S.A.) for 1 h at 90°C. Then this reaction mixture was evaporated under nitrogen. The residue was applied to TLC plates (20 x 20 cm; silica gel 60, 0.25 mm layer, Merck, Darmstadt) using 50 μl benzene. The plates were preimpregnated with methylene chloride-methanol (1:1). The plates were developed in a solution of methylene chloride-methanol-benzene (45:45:5) to a height of 17 cm. Digitoxin HBF