Digoxin is excreted primarily by the kidney as an unchanged glycoside. Only in the first hours after oral absorption is there a chloroform-insoluble metabolite fraction detectable in the urine, this phenomenon being first described in 1972 by Dwenger-Haberland (Dwenger and Haberland, 1972). The polar fraction in urine increases to a maximum 4 h after ingestion of the glycoside. At this time, half of the renally excreted amount is not extractable with chloroform. Ten hours after, this fraction decreases to a very low level. The purpose of this study is to describe the time-dependent occurrence of the polar fraction after digoxin, and to compare it with the behavior of digitoxin. Further, we have carried out some analytic work in the polar metabolites of digoxin.

Methods and Materials

Experiments were performed on healthy male volunteers, aged 41-60 years. Three persons received orally 21-22-3H-digitoxin (~100 μCi) (Haberland and Maerten, 1969) plus 1.4 mg inactive drug and six persons 12-α-3H-digoxin (~100 μCi) plus 1.6 mg inactive drug. The glycosides were administered in a volume of 4 ml 30% vol/vol ethanol plus 100 ml water. The administration was always 3 h after the last meal.

Urine collections began 30 min after dosing and continued in 1-h intervals up to 12 h, thereafter in longer intervals, terminating after 72 h; 0.2 ml of each urine sample was added to scintillation fluid and directly counted. Following this, the whole urine sample was divided into smaller aliquots, each extracted three times with twice the volume of ethyl acetate. The extract was evaporated to dryness, redissolved in 10 ml methanol, and aliquots of the extract and the nonextractable fraction counted. The complete water-soluble residue was applied to an Amberlite® XAD-2 column. After collection of a high polar H₂O fraction, the main ³H activity could be eluted by ethanol (Fig. 5.1). The ethanol fraction was cleaned up on Sephadex® LH-20 several times.

After evaporating to dryness, an aliquot was applied to TLC plates whereas most of the residue was dissolved in 5 ml acetate buffer (pH 5.1) and incubated with glucuronidase sulfatase for 48 h at 37°C. Tritium activity was determined, and the whole solution extracted three times with chloroform. The evaporated chloroform
layers were redissolved in methanol and aliquots used for measuring radioactivity and TLC. Aliquots of the remaining aqueous layers were taken for counting.

For further elucidation of the polar fraction, a concentrated aliquot was applied with a pyrophosphate-HCl buffer pH 8.1 to a paper electrophoresis system; radioactivity was located by scanning. TLC was performed on silica gel plates 0.25 mm (Merck). Solvent systems: 1. chloroform methanol 92:8; 2. ethyl acetate; 3. chloroform-methanol-water 64:31:5. The radioactive zones were located with the Berthold scanner II and compared with reference substances after staining with trichloracetic acid. Mass spectrometry was performed on a Varian MAT-311-A instrument equipped with a field desorption electron impact combination ion source.

Results

As already described by Dwenger and Haberland (1972), the water-soluble digoxin metabolites in urine are excreted within the first 24 h. After reaching a maximum between the 2nd and 6th h, the fraction decreases rapidly to a very low level, whereas the total $^3$H excretion increases further. Figure 5.2 shows the time-dependent relation within the interval from 0-8 h and 0-24 h. Between 8 and 24 h after dosing, the total amount of excreted radioactivity increases averagely from 20%-30% of the dose, whereas the water-soluble fraction only shows small increments within the same time interval.

In different persons, the amount of water-soluble products varied in a range from 6%-13% of the dose. However, there was a remarkable variation in the same person, as seen in Figure 5.2. In the first experiment, 10% of the dose was excreted in form