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GLUCURONIDATION OF DIGITALIS GLYCOSIDES BY RAT LIVER MICROSOMES: STIMULATION BY SPIRONOLACTONE AND PREGNENOLONE-16α-CARBONITRILE

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Abstract—In the present studies, glucuronidation of [^{3}H]digoxigenin monodigitoxoside and [^{3}H]digitoxigenin monodigitoxoside has been investigated *in vitro* using rat liver microsomes. During a 40-min incubation period, the conjugation of digitoxigenin monodigitoxoside was five times greater than of digoxigenin monodigitoxoside at a substrate concentration of 5 μM and three times greater at a 20 μM substrate concentration. No difference in conjugation rates was observed between male and female rats, with either substrate. Pretreatment with spironolactone (100 mg/kg/day for 3 days) stimulated the conjugation of both substrates in females, but no significant changes were observed in males with either substrate. Pretreatment (75 mg/kg/day) with pregnenolone-16α-carbonitrile (PCN) caused enhanced glucuronidation of both substrates in both sexes. The conjugation rates in the PCN-pretreated rats were approximately twice those of the spironolactone-pretreated rats. The possible role of this increased conjugation in the protective effect of spironolactone and PCN against digitalis toxicity is discussed.

The major metabolic pathways for the metabolism of digoxin and digitoxin have been known for many years (see Refs. 1 and 2 for reviews). Each glycoside is metabolized by stepwise removal of the three digitoxose sugars attached at the carbon-3 position of the steroid nucleus, yielding, respectively, for each drug, the bisdigitoxoside, the monodigitoxoside and the genin. Until recently it was assumed that sulfate and glucuronide conjugation occurred with digoxigenin and digitoxigenin (or the related metabolites, epidigoxigenin and epidigitoxigenin). In recent years, however, evidence has accumulated which suggests that the primary conjugation reaction is the formation of glucuronide conjugates [3–6]. Furthermore, it was demonstrated that for digitoxin this glucuronidation reaction occurs almost exclusively with the monodigitoxoside both *in vivo* [6] and *in vitro* [5]. Since these glucuronide conjugates are rapidly excreted into the bile [3, 6], this pathway appears to be an important detoxification step for the cardiac glycosides.

The ability of spironolactone to protect rats against otherwise toxic doses of digitoxin is related to a markedly enhanced formation of these glucuronide conjugates [3, 6]. While there is little doubt that this protective effect of spironolactone is due at least in part to a stimulation of the glycolytic pathway for digitoxin metabolism, it is not clear what role enhanced glucuronidation may play. Richards and Lage [7] have reported recently that rat liver homogenates from male rats pretreated with spironolactone exhibited greatly increased glucuronide formation with digitoxigenin monodigitoxoside. Although digoxin is metabolized by the same pathways as digitoxin [8], it is not known if the rates of glucuronidation are similar for the two glycosides or if spironolactone pretreatment can stimulate the conjugation of digoxigenin monodigitoxoside to the same extent as that of digitoxigenin monodigitoxoside.

The present investigations have been undertaken, therefore, to compare the rates of glucuronidation in microsomal preparations from male and female rats pretreated with spironolactone. Since pregnenolone-16α-carbonitrile (PCN) has been shown to be a more powerful inducer than spironolactone of digitoxin metabolism [9], the ability of these two drugs to stimulate glucuronidation has been compared. It was also of interest to compare the rates of conjugation of digoxigenin monodigitoxoside and digitoxigenin monodigitoxoside and to determine if pretreatment with the above enzyme-inducing agents stimulates the glucuronidation of digoxigenin monodigitoxoside in the same manner as digitoxigenin monodigitoxoside.

MATERIALS AND METHODS

Drugs. The tritiated substrates (digoxigenin monodigitoxoside and digitoxigenin monodigitoxoside) were prepared from [^{3}H]digoxin and [^{3}H]digitoxin (New England Nuclear, Boston, MA). One mCi of each compound was incubated separately (37° for 1 hr) in 2 ml of dilute HCl (pH 2.0). At the end of the incubation period, carrier compounds were added to both the [^{3}H]digoxin mixture (10 nmoles each of digoxin, the bis- and monodigitoxosides, and digoxigenin) and to the [^{3}H]digitoxin mixture (10 nmoles each of digitoxin, the bis- and monodi-

gitoxosides, and digitoxigenin). Ethanol (5 ml) was added and the mixture was extracted three times with 5 ml chloroform. The chloroform fractions were combined and evaporated to dryness at 40° under nitrogen. The residue was transferred to a small vial with chloroform, evaporated, and then redissolved in 100 μl ethanol. The high-pressure liquid chromatography (h.p.l.c.) method described previously [10] was used to isolate the digoxigenin monodigitoxoside and digitoxigenin monodigitoxoside. The radiochemical purity of both of these substrates was greater than 95 per cent. For each glycoside, non-radioactive compound was added to bring each drug to a final specific activity of either 40 mCi/mmole or 10 mCi/mmole. Spironolactone (Sigma Chemical Co., St. Louis, MO) and pregnenolone-16α-carbonitrile (The Upjohn Co., Kalamazoo, MI) were prepared in 2 per cent Tween 80 in water. The non-radioactive derivatives of digoxin and digitoxin were purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN). Type I β-glucuronidase was obtained from the Sigma Chemical Co.

Animals. Male and female Sprague–Dawley rats (150–200 g) were purchased from Flow Laboratories (Dublin, VA). Spironolactone (100 mg/kg/day), pregnenolone-16α-carbonitrile (75 mg/kg/day) or vehicle (2 per cent Tween 80 in water) was injected i.p. (2 ml/kg) into the appropriate animal on 3 consecutive days. Twenty-four hours after the last pretreatment the rats were killed by cervical dislocation and the livers quickly removed and placed in ice-cold buffer solution.

Preparation and incubation of microsomes. Four grams of liver were weighed and placed in 16 ml of ice-cold buffer (50 mM Tris, 114.9 mM NaCl and 5.9 mM KCl). The livers were homogenized using a motor-driven Teflon-on-glass Potter homogenizer. Washed microsomes were prepared by the method of Kutt and Fouts [11]. Microsomal protein was assayed using the method of Lowry *et al.* [12]. The complete incubation volume of 5.0 ml consisted of 10 mg microsomal protein, 10 mg uridine-5′-diphosphoglucuronic acid (UDPGA) and either 25 nmoles or 100 nmoles of the appropriate substrate (either [^{3}H]digoxigenin monodigitoxoside or [^{3}H]digitoxigenin monodigitoxoside). A preincubation period was used in which the tritiated substrate was added to the microsomal preparation and incubated at 37° for 20 min prior to the addition of UDPGA. After the addition of UDPGA, 0.5-ml aliquots were withdrawn at several time intervals (2.5, 5, 10, 20 and 40 min) and assayed for glucuronide conjugates as described below.

Isolation of glucuronide conjugates. Each aliquot from the microsomal incubation mixtures was placed in a 15 ml polypropylene centrifuge tube containing 5 ml of 50 per cent ethanol and water. The sample was mixed, centrifuged and decanted. The pellet was extracted twice more with 5 ml of 50 per cent ethanol and the three supernatant fractions were combined. The combined aqueous–alcohol fraction was extracted three times with 15 ml chloroform. The chloroform fractions (containing the original substrate) were combined, evaporated to dryness, and then assayed for total radioactivity. The remaining aqueous–alcohol fraction (containing conjugation products) was placed in a volumetric flask and an aliquot was withdrawn for assay of total radioactivity. Separate aliquots (one third of the total amount) of this aqueous–alcohol fraction were evaporated to dryness and then either incubated with β-glucuronidase as described previously [5] or applied to the h.p.l.c. system described below.

The h.p.l.c. procedure reported previously [10] was modified to permit the separation of the glucuronide conjugates and the initial substrates in the same sample. The specific conditions for this separation are presented with the chromatogram (see Fig. 1). The fractions collected from the h.p.l.c. procedure were assayed to determine the amount of radioactivity which eluted along with the glucuronide conjugates and with the original substrates. An aliquot (one half of the total amount) of eluates numbered 2 and 4 on the chromatogram (Fig. 1) was incubated with β-glucuronidase as reported previously [6] and then extracted with chloroform as before. The radioactivity in the polar and non-polar fractions was assayed to determine the extent of the conversion of the glucuronide conjugate to the original substrate. An aliquot of the chloroform-soluble fractions was also applied to h.p.l.c. to confirm that the radioactivity liberated by the action of β-glucuronidase was still in the form of the original substrate (i.e. either digoxigenin monodigitoxoside or digitoxigenin monodigitoxoside).

Statistical analysis. Samples were counted for radioactivity in 15 ml of ACS scintillation mixture (Amersham/Searle, Arlington Heights, IL) using an ambient temperature Beckman model LS 250 liquid scintillation spectrometer. Quench corrections were performed using an external standard. Disintegration rates (d.p.m.) were converted to units of nanomoles based upon the initial specific activity of the substrate and making the assumption that no label had been lost from the steroid nucleus during the conversion of the parent compound to the glucuronide conjugate. A randomized complete block analysis of variance was performed at each time period. In all groups where the analysis of variance indicated significant differences ($P < 0.05$), the Student–Newman–Keuls test [13] was performed on all possible combinations at each time period. Significant differences ($P < 0.05$) between the means are noted in Results. The data were also subjected to regression analysis. The best regression line was determined for each sample over the period of time during which the reaction was linear (usually 0–20 min). These individual conjugation rates were then used to calculate the mean and standard error for each group. An analysis of variance was performed on these data as described above.

RESULTS

The tritiated substrates (digoxigenin monodigitoxoside and digitoxigenin monodigitoxoside) were purified by h.p.l.c. to a final radiochemical purity of 99 per cent; the majority of the remaining radioactivity was chromatographically identical to the respective genin. Separation of the conjugation products from the starting substrate was accomplished by two procedures: solvent–solvent extrac-

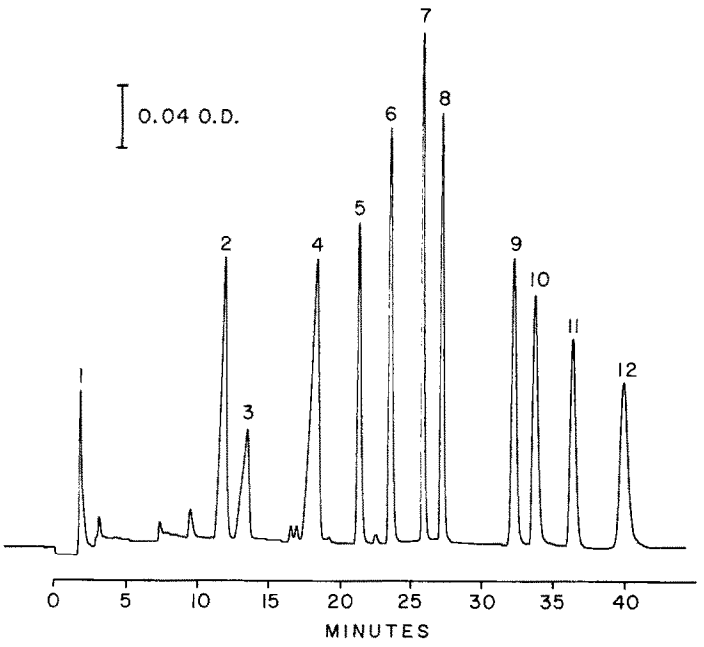

Fig. 1. Isolation of digoxin, digitoxin and their metabolites by high-pressure liquid chromatography. The column was a Hibar-II prepacked column (EM Laboratories, Elsmford, NY) containing LiChrosorb RP-18 packing material. The mobile phase (flow rate 2.5 ml/min) consisted of a linear gradient of 100 per cent water to 40 per cent acetonitrile in water at 4 per cent/min. The u.v. motor was set at 220 nm with attenuation at 0.4 a.u.f.s. A reference mixture containing 15 nmoles of each compound was injected in 50 μl ethanol. Peak identities: 1 = solvent front; 2 = glucuronide of digoxigenin monodigitoxoside; 3 = non-radioactive unknown; 4 = glucuronide of digitoxigenin monodigitoxoside; 5 = digoxigenin; 6 = digoxigenin monodigitoxoside; 7 = digoxigenin bisdigitoxoside; 8 = digoxin; 9 = digitoxigenin; 10 = digitoxigenin monodigitoxoside; 11 = digitoxigenin bisdigitoxoside; and 12 = digitoxin.

tion and high-pressure liquid chromatography. The extraction procedure has been used extensively in previous studies [5, 6, 14] and is based upon partitioning of the conjugation products into an aqueous–alcohol fraction and the unconjugated glycosides into a chloroform fraction. The second procedure, which has not been previously reported, involves isolation of the glucuronide conjugates by h.p.l.c. (Fig. 1). The extraction procedure described above did not distinguish between glucuronide conjugates and other water-soluble conjugates since the aqueous–alcohol fraction also contained sulfate conjugates and other highly polar metabolites. The h.p.l.c. method, on the other hand, does allow the glucuronide conjugates to be isolated and quantitated separately. It is also possible with this h.p.l.c. method to distinguish between the glucuronides formed from digoxin and digitoxin derivatives. Thus, this h.p.l.c. method may prove useful in distinguishing between the glucuronides of digoxigenin monodigitoxoside and digitoxigenin monodigitoxoside formed *in vivo*. In the present studies, quantitation of the glucuronide conjugates was accomplished by determining the amount of radioactivity associated with each area of the chromatogram. When digoxigenin monodigitoxoside was the starting substrate, over 95 per cent of the radioactivity in the polar fraction was recovered in peak 2 (Fig. 1). With digitoxigenin monodigitoxoside, over 96 per cent of the radioactivity was found to be associated with peak 4 (Fig. 1).

In order to confirm that the reaction being studied involved glucuronidation of the monodigitoxosides, aliquots of the water-soluble metabolites isolated by either the extraction technique or by h.p.l.c. were incubated with β-glucuronidase as described previously [5]. For all samples this incubation procedure resulted in conversion of over 96 per cent of the radioactivity from the water-soluble fraction to the chloroform-soluble fraction. Analysis of these chloroform-soluble fractions by h.p.l.c. indicated that these non-polar compounds released by the action of β-glucuronidase were chromatographically identical to the initial substrates (i.e. either digoxigenin monodigitoxoside or digitoxigenin monodigitoxoside, respectively). When 1 mM saccharo-1,4-lactone (a specific inhibitor of β-glucuronidase) was included in the same incubation with β-glucuronidase, only about 5 per cent of the radioactivity was converted from the water-soluble fraction to the chloroform-soluble fraction. These results suggest that the only reaction occurring in these microsomal preparations was glucuronidation of the parent substrate.

The conjugation of digoxigenin monodigitoxoside was not significantly different in male and female rats (Table 1). Spironolactone pretreatment stimulated conjugation in female, but not in male, rats. Pretreatment with PCN caused enhanced conjugation in both sexes. Conjugation rates were 2–3 times greater in animals pretreated with PCN than in animals pretreated with spironolactone.

Table 1. Effects of spironolactone and PCN pretreatment on the conjugation of digoxigenin monodigitoxoside by rat liver microsomes*

Time (min)	Conjugation of digoxigenin monodigitoxoside† Male Control	Male Spironolactone	Male PCN	Female Control	Female Spironolactone	Female PCN
2.5	0.38 ± 0.06	0.44 ± 0.03‡	0.99 ± 0.15§	0.33 ± 0.02	0.84 ± 0.14§	1.08 ± 0.05§
5	0.45 ± 0.02	0.63 ± 0.03‡	1.38 ± 0.21§	0.41 ± 0.03	1.03 ± 0.13§	1.75 ± 0.07§
10	0.58 ± 0.05	0.87 ± 0.10‡	2.01 ± 0.28‡§	0.57 ± 0.04	1.59 ± 0.23§	3.06 ± 0.11§
20	0.85 ± 0.16	1.38 ± 0.21‡	3.38 ± 0.45‡§	0.75 ± 0.08	2.77 ± 0.40§	5.89 ± 0.22§
40	1.43 ± 0.20	2.06 ± 0.23‡	6.83 ± 1.04‡§	1.26 ± 0.08	4.66 ± 0.61§	10.22 ± 0.41§

* Aliquots (0.5 ml) were withdrawn from incubation mixtures (5.0 ml) containing 25 nmoles substrate, 15 μmoles UDPGA and 10 mg microsomal protein.
† Data are expressed as means ± S.E. in nmoles of substrate conjugated.
‡ $P < 0.05$, with respect to corresponding female group.
§ $P < 0.05$, with respect to corresponding control group.

Table 2. Effects of spironolactone and PCN pretreatment on the conjugation of digitoxigenin monodigitoxoside by rat liver microsomes*

Time (min)	Conjugation of digitoxigenin monodigitoxoside† Male Control	Male Spironolactone	Male PCN	Female Control	Female Spironolactone	Female PCN
2.5	1.63 ± 0.42	1.84 ± 0.14	4.18 ± 0.71‡	1.17 ± 0.05	2.07 ± 0.27‡	4.68 ± 0.23‡
5	2.16 ± 0.23	2.58 ± 0.12	4.89 ± 0.82‡	1.70 ± 0.06	3.09 ± 0.45‡	6.85 ± 0.29‡
10	3.33 ± 0.35	4.26 ± 0.22	7.57 ± 0.83‡§	2.62 ± 0.17	5.64 ± 0.88‡	10.99 ± 0.52‡
20	5.20 ± 0.54	6.01 ± 0.21	12.31 ± 0.59‡	4.24 ± 0.32	7.85 ± 1.02‡	13.23 ± 0.72‡
40	8.33 ± 0.78	10.19 ± 0.65	13.79 ± 0.60‡	6.58 ± 0.62	8.40 ± 0.30‡	12.46 ± 0.70‡

* Aliquots (0.5 ml) were withdrawn from incubation mixtures (5.0 ml) containing 25 nmoles substrate, 15 μmoles UDPGA and 10 mg microsomal protein.
† Data are expressed as means ± S.E. in nmoles of substrate conjugated.
‡ $P < 0.05$, with respect to corresponding control group.
§ $P < 0.05$, with respect to corresponding female group.

Table 3. Effects of spironolactone and PCN pretreatment on the conjugation of digoxigenin monodigitoxoside by rat liver microsomes*

Time (min)	Conjugation of digoxigenin monodigitoxoside† Male Control	Male Spironolactone	Male PCN	Female Control	Female Spironolactone	Female PCN
2.5	2.45 ± 0.38	2.24 ± 0.10‡	2.55 ± 0.16	1.74 ± 0.07	1.65 ± 0.14	3.41 ± 0.37§
5	3.96 ± 0.85	2.49 ± 0.10	3.18 ± 0.29‡	2.37 ± 0.33	2.24 ± 0.27	4.55 ± 0.37§
10	3.95 ± 0.81	2.64 ± 0.13	4.90 ± 0.59	2.18 ± 0.10	3.70 ± 0.45§	6.63 ± 0.43§
20	4.71 ± 0.90‡	4.17 ± 0.15‡	8.29 ± 1.09‡§	2.34 ± 0.09	6.34 ± 0.75§	12.61 ± 0.94§
40	4.49 ± 0.61‡	5.89 ± 0.28‡	16.02 ± 2.19§	3.16 ± 0.11	11.05 ± 1.45§	21.96 ± 1.09§

* Aliquots (0.5 ml) were withdrawn from incubation mixture (5.0 ml) containing 100 nmoles substrate, 15 μmoles UDPGA and 10 mg microsomal protein.
† Data are expressed as means ± S.E. in nmoles of substrate conjugated.
‡ $P < 0.05$, with respect to corresponding female group.
§ $P < 0.05$, with respect to corresponding control group.

Table 4. Effects of spironolactone and PCN pretreatment on the conjugation of digitoxigenin monodigitoxoside by rat liver microsomes*

Time (min)	Conjugation of digioxigenin monodigitoxoside† Male Control	Male Spironolactone	Male PCN	Female Control	Female Spironolactone	Female PCN
2.5	4.18 ± 0.23‡	3.33 ± 0.24	5.24 ± 0.43	2.87 ± 0.25	2.85 ± 0.19	6.34 ± 0.52§
5	4.25 ± 0.18‡	4.27 ± 0.36	7.89 ± 0.86‡§	3.24 ± 0.15	4.15 ± 0.30§	10.54 ± 0.42§
10	5.47 ± 0.44	5.87 ± 0.25	11.50 ± 1.02§	4.49 ± 0.21	6.17 ± 0.41§	14.47 ± 1.03§
20	8.89 ± 1.06	10.11 ± 0.38	17.97 ± 1.40‡§	7.26 ± 0.29	10.52 ± 0.85§	24.63 ± 1.68§
40	12.03 ± 1.25	14.20 ± 0.76	30.91 ± 2.79‡§	9.99 ± 0.40	16.81 ± 1.19§	41.91 ± 2.71§

* Aliquots (0.5 ml) were withdrawn from incubation mixture (5.0 ml) containing 100 nmoles substrate, 15 μmoles UDPGA and 10 mg microsomal protein.
† Data are expressed as means ± S.E. in nmoles of substrate conjugated.
‡ $P < 0.05$, with respect to corresponding female group.
§ $P < 0.05$, with respect to corresponding control group.

Table 5. Rates of conjugation of digoxigenin monodigitoxoside and digitoxigenin monodigitoxoside*

Treatment	(25 nmoles/5.0 ml) Digoxigenin monodigitoxoside	(25 nmoles/5.0 ml) Digitoxigenin monodigitoxoside	(100 nmoles/5.0 ml) Digofigenin monodigitoxoside	(100 nmoles/5.0 ml) Digitoxigenin monodigitoxoside
Male				
Control	0.027 ± 0.002	0.195 ± 0.010	0.101 ± 0.021†‡	0.277 ± 0.068
Spironolactone	0.035 ± 0.003†	0.237 ± 0.017	0.109 ± 0.002†	0.387 ± 0.024
PCN	0.135 ± 0.016†‡	0.474 ± 0.019‡	0.333 ± 0.053†‡	0.681 ± 0.045†‡
Female				
Control	0.024 ± 0.005	0.174 ± 0.017	0.021 ± 0.007	0.257 ± 0.002
Spironolactone	0.112 ± 0.015‡	0.340 ± 0.049‡	0.270 ± 0.032‡	0.433 ± 0.034‡
PCN	0.275 ± 0.005‡	0.478 ± 0.024‡	0.528 ± 0.029‡	1.008 ± 0.065‡

* Rates of conjugation were determined from regression analysis of the data in Tables 1–4. Data are expressed as means ± S.E. in nmoles/min/10 mg microsomal protein.
† $P < 0.05$, with respect to corresponding female group.
‡ $P < 0.05$, with respect to corresponding control group.

As shown in Table 2, the conjugation of digitoxigenin monodigitoxoside was approximately 5 times greater than that observed with digoxigenin monodigitoxoside (Table 1). No differences were observed in the conjugation of digitoxigenin monodigitoxoside between male and female rats (except the 10 min PCN-pretreated group). PCN pretreatment stimulated conjugation in both sexes, but only the female group were stimulated by spironolactone pretreatment. This spironolactone-pretreated female group, however, was still not significantly different from either the male control group or the male group pretreated with spironolactone. In general, the stimulatory effect of both spironolactone and PCN was found to be greater when digoxigenin monodigitoxoside was the substrate than when digitoxigenin monodigitoxoside was used.

The results obtained at the 20 μM substrate concentration are shown in Tables 3 and 4. The differences observed between digoxigenin monodigitoxoside and digitoxigenin monodigitoxoside at the lower substrate concentration (Tables 1 and 2) were not as great at the higher concentration. Spironolactone pretreatment did not stimulate conjugation in male rats but did cause significant increases in female rats. PCN pretreatment resulted in enhanced conjugation in both sexes.

The results shown in Tables 1–4 are summarized in Table 5 which illustrates the rates of conjugation in the various groups as determined by regression analysis of the linear portions of the curves obtained by plotting the amount of conjugation products formed against time.

DISCUSSION

Digoxin and digitoxin are metabolized by similar pathways: stepwise cleavage of two of the three digitoxose sugars to yield the bisdigitoxosides and the monodigitoxosides, respectively. These monodigitoxosides are then conjugated with glucuronic acid to form highly polar glucuronide conjugates. In previous studies with digitoxin, indirect evidence has been presented which suggests that this polar fraction

is composed entirely of the glucuronide conjugate of the monodigitoxoside [3–6]. In the present studies, the polar fraction from the incubation of each of the tritiated monodigitoxosides has been analyzed by high-pressure liquid chromatography (h.p.l.c.). For each substrate, only a single radioactive peak was detected using this h.p.l.c. technique. In each case, this radioactivity was almost entirely converted back to the initial substrate by the action of β-glucuronidase. This action of β-glucuronidase was almost entirely blocked by saccharo-1,4-lactone (a specific inhibitor of β-glucuronidase). If other conjugation products were formed (e.g. sulfates), these would be expected to behave differently from the glucuronides in the h.p.l.c. procedure and would not be totally cleaved by β-glucuronidase. This evidence strongly suggests that, under the conditions of the present studies, the only *in vitro* metabolism which occurs with these glycosides is the formation of glucuronide conjugates.

The conversion of digoxin to metabolic products is considerably less extensive than that of digitoxin. The results of the present studies indicate that the rate of conjugation of digitoxigenin is 3–6 times greater than that of digoxigenin monodigitoxoside. It thus appears that the relative rates of glucuronidation of these two glycosides follow a pattern similar to the glycolytic pathway of the parent compounds. These differences were less apparent after pretreatment with either spironolactone or PCN since the conjugation of digoxigenin monodigitoxoside was stimulated to a greater extent than that of digitoxigenin monodigitoxoside. For both substrates the stimulation of glucuronidation was significantly higher with PCN than with spironolactone pretreatment. These results are consistent with previous studies which indicated that PCN has a greater stimulatory effect than spironolactone on the glycolytic pathway for digitoxin metabolism [9] and may be related to observations that PCN is considerably more potent than spironolactone in preventing digitoxin toxicity in rats. In the present studies no sex differences were observed in the glucuronidation of either glycoside in untreated rats. Spironolactone pretreatment stimulated the formation of conjugation products in females but there was no stimulation in males. PCN pretreatment caused enhanced conjugation in both males and females. This change was similar in both sexes when digitoxigenin monodigitoxoside was the substrate but with digoxigenin monodigitoxoside the stimulatory effect of PCN was much greater in the females than in the males. These data thus suggest that both of these drugs stimulate the glucuronidation process in females to a significantly greater degree than in males. Previous studies have demonstrated that spironolactone stimulates certain oxidative pathways in female rats but not in male rats [15].

Although the present studies demonstrate that pretreatment with either spironolactone or PCN markedly enhances the glucuronidation pathway for digitalis glycosides, it is not clear what role this stimulation plays in preventing digitalis toxicity in rats. Spironolactone pretreatment prevents digitoxin toxicity in both male and female rats [16, 17] but this pretreatment appears to stimulate glucuronidation only in females. However, spironolactone pretreatment does stimulate the glycolytic pathway of digitoxin metabolism in male rats [9] and also increases biliary excretion of digitalis glycosides by a mechanism other than increased biotransformation [16]. These results suggest that the stimulation of glucuronidation by spironolactone and PCN may be less important in preventing digitoxin toxicity than enhanced glycolytic metabolism and biliary excretion of unconjugated compounds.

In conclusion, the present studies have shown that glucuronidation of digitoxigenin monodigitoxoside by rat liver microsomes occurs to a much greater extent than of digoxigenin monodigitoxoside. Although no sex differences were observed with either substrate in untreated animals, pretreatment with spironolactone was found to stimulate conjugation of both substrates in females but not in males. PCN stimulates glucuronidation in both sexes and this stimulation is much greater than that observed with spironolactone. It is doubtful, however, that stimulation of this metabolic pathway is involved in the protective effect of these drugs against digitalis toxicity.

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