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Hydrolysis of digoxin by acid

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Predictable hydrolysis of [^{3}H]digoxin-12α occurred *in vitro* with incubation in HCl or gastric juice. Hydrolysis varied with pH, time, temperature and agitation. Digoxin, the bis- and mono-digitoxosides of digoxigenin and digoxigenin were separated by silica gel thin-layer chromatography using chloroform–ethyl acetate–glacial acetic acid (25:25:1 v/v) and were quantitated by liquid scintillation spectrometry. Hydrolysis with incubation at 37° and pH 3 for 90 min was minimal, but increased with increasing acidity until >70% was hydrolysed at pH 1·2 after 30 min and >96% after 90 min incubation. At pH 0·9, 87% was hydrolysed after 30 min. *In vitro* hydrolysis in gastric fluid was slightly less than in HCl at the same pH. A volunteer was given 150 μCi[^{3}H]digoxin-12α by nasogastric tube during a pentagastrin infusion when gastric pH was 0·94. He remained on his left side and samples were aspirated at intervals and immediately neutralized. Ethanol–chloroform 50:50 (v/v) extracts of the gastric fluid aspirated after 90 min and of all the urine specimens collected for 5 days were applied to a DEAE Sephadex LH-20 column. The radioactivity appeared in a single peak as digoxigenin in the 90 min gastric aspirate and in all urine specimens. Extensive intragastric hydrolysis of digoxin may occur under conditions of maximum acid output.

Beermann, Hellstrom & Rosen (1972) reported that an average of 7% (2–14%) of [^{3}H]digoxin-12α given to man in the fasting state was hydrolysed in the stomach to digoxigenin and its mono- and bis-digitoxosides in 55 min. The pH of the aspirated gastric samples ranged from 1·6 to 3·2. *In vitro* incubation of digoxin in acid with pH in the range of 3–10 produced no hydrolysis, but incubation at pH 1 and 2 showed that 21 and 1% respectively of the glycoside had been transformed to digoxigenin and its mono- and bis- digitoxosides within 15 min. After 2 h the corresponding values were 99 and 21%. It is, therefore, possible that with maximum hydrogen ion secretion, intragastric hydrolysis of digoxin could be extensive, and in part explain the wide variation in the percentage of digoxin and dihydrodigoxin converted to their glycoside metabolites (0–42%) reported by Clark & Kalman (1974).

We have studied the characteristics of the hydrolysis of digoxin by acid *in vitro*, in relation to pH, time, temperature and agitation and compared the effect of gastric juice with that of hydrochloric acid and have also studied intragastric hydrolysis at maximum acid secretion induced by pentagastrin infusion (Charles, Sugden & others, 1976).

MATERIALS AND METHODS

Materials

Pre-coated silica gel chromatograms (0·1 mm, 20 cm × 20 cm) (Eastman Kodak) were developed using an Eastman Chromagram Developing Apparatus 6071. Incubation was performed using a Dubnoff Metabolic Shaking Incubator (GCA-Precision Scientific Co., Chicago, Illinois). Radioactivity was counted with a Beckman LS-330 liquid scintillation spectrometer (Beckman Instruments Inc., Fullerton, California).

Reagent grade hydrochloric acid, sodium hydroxide, glacial acetic acid and trichloroacetic acid were purchased from Fisher Scientific Co., Montreal, Quebec; ethyl acetate, methylene chloride, methanol and chloroform from Anachemia Chemicals Ltd., Montreal; chloramine-T and 2-chlorotriethylamine hydrochloride from Eastman Kodak Co., Rochester, New York, and Sephadex LH-20 from Pharmacia Fine Chemicals, AB Uppsala, Sweden. The liquid scintillation cocktails, Aquasol and Riafluor, were purchased from New England Nuclear, Boston, Mass. [^{3}H]Toluene 0·6 μCi mg^{-1} (lot H274 Amersham/Searle Corp., Arlington Heights, Ill.) was used as an internal standard.

Burroughs-Wellcome Co., Research Triangle Park, North Carolina, kindly supplied digoxin and [^{3}H]digoxin-12α (lot Mu 20–75, 2·36 mCi mg^{-1}). [^{3}H]digoxin-12α with a specific activity of 23 mCi mg^{-1} was obtained from New England Nuclear, Boston, Mass. (NEN 853–140). Digoxigenin-bis-digitoxoside, and digoxigenin-mono-digitoxoside and digoxigenin were purchased from Boehringer-Mannheim, West Germany, lots 61016, 7681 and 154182, respectively.

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Methods

Ten μg of digoxin and 0·04 μg of [^{3}H]digoxin-12α (200 000 d min^{-1}) were added to each of a series of tubes and the solvent evaporated. Hydrochloric acid 0·5 ml, or 1·5 ml human gastric juice of known pH, was added to each tube and incubated either at room temperature or 37°, with or without shaking, for varying periods of time. Neutralization was then effected with an equivalent volume of sodium hydroxide of appropriate pH, and the drugs extracted three times with 2 ml of methylene chloride. The extracts were evaporated with a stream of air until about 0·1 ml remained and then spotted on chromatographic plates activated at 110° for 30 min. Five μg each of digoxin, digoxigenin-bis-digitoxoside, digoxigenin-mono-digitoxoside and digoxigenin were applied as standards. The chromatograms were developed twice in the same direction using chloroform–ethyl acetate–glacial acetic acid (25:25:1 v/v) as solvent, sprayed lightly with 3% chloramine T-trichloroacetic acid solution (Waldi, 1959) and heated at 110° for 8 min.

Digoxin and the hydrolysis products were visualized with ultraviolet light at 350 nm and the spots were scraped into glass scintillation vials containing 3·0 ml distilled water and 10 ml Aquasol and counted. [^{3}H]Toluene was then added to each vial as an internal standard and a correction made for quenching. The amounts of digoxin and hydrolysis products were expressed as d min^{-1} and the percentages calculated.

Liquid scintillation counting was performed with a maximum error of 2% at 95% confidence limits.

In order to confirm the results of digoxin hydrolysis obtained *in vitro*, a 25 year old healthy male volunteer who gave informed consent, received 250 μg of unlabelled digoxin (Lanoxin lot No. 401-F) and 150 μCi (70 μg) of [^{3}H]digoxin-12α through a nasogastric tube, 30 min after the beginning of an intravenous pentagastrin infusion of 6 μg kg^{-1} h^{-1}. He was placed on his left side to retard gastric emptying and gastric aspirates of 10, 5, 5, 7 and 230 ml were collected at 0, 20, 40, 60, and 90 min after the digoxin was administered. No attempt was made to empty the stomach, except at 90 min. Aliquots to be used for chromatography were immediately titrated to pH 8–10 with N sodium hydroxide. Urine was collected serially for 5 days. The same volunteer ingested 150 μCi (70 μg) [^{3}H]digoxin-12α several weeks later without a pentagastrin infusion. Urine was again collected serially for 5 days and specimens were analysed for digoxin and metabolites by the same methods.

Samples of gastric juice and urine were extracted three times with chloroform–ethanol (1:1 v/v). The volume of solvent used was double the amount of urine and three times the amount of gastric juice. The solvent was evaporated to leave a volume of 0·1 ml for application to thin layer plates. Extracts of gastric juice and urine were also applied to a DEAE Sephadex LH-20 column, eluted with chloroform–methanol 85:15 (v/v), and the fractions counted in Riafluor (Sugden, Ahmed & Gault, 1976).

RESULTS

The reproducibility of the thin-layer chromatographic system used was excellent. Mean R_F values for 16 runs with s.d. were for digoxin 0·15 (0·01), bis-digitoxosides of digoxigenin 0·24 (0·01), mono-digitoxoside of digoxigenin 0·38 (0·01), and digoxigenin 0·51 (0·01).

The influence of pH and time on the rate of hydrolysis of digoxin to digoxigenin and its mono- and bis-digitoxoside with incubation in hydrochloric acid at 37° is shown in Table 1 and Fig. 1. Hydrolysis of digoxin was slight at pH 3, even after 90 min (6%); however, for a given incubation time,

Table 1. *% Radioactivity as [^{3}H]digoxin and metabolites after incubation of [^{3}H]digoxin-12α at 37° with agitation for 10 to 90 min with hydrochloric acid at pH 0·9 to 3: means of 4 determinations with s.d.*

Duration of incubation	10 min					30 min							90 min					
pH	0·9	1·2	1·5	1·8	2·0	0·9	1·2	1·5	1·8	2·0	2·5	3·0	1·2	1·5	1·8	2·0	2·5	3·0
Digoxin	49·2 (0·9)	66·7 (9·5)	74·0 (1·1)	90·7 (2·8)	92·9 (2·1)	13·3 (0·9)	28·8 (5·8)	51·8 (1·9)	73·9 (3·0)	84·0 (3·0)	93·4 (1·9)	97·7 (1·0)	3·7 (1·0)	19·4 (0·6)	49·5 (0·5)	62·1 (3·5)	85·8 (3·0)	94·1 (1·4)
Digoxigenin bis-digitoxoside	18·7 (0·7)	10·6 (1·7)	7·2 (0·7)	4·5 (1·0)	3·4 (1·3)	14·9 (1·0)	16·3 (2·7)	14·9 (0·6)	12·5 (1·6)	7·6 (2·5)	5·1 (2·0)	0·9 (0·5)	3·7 (1·4)	14·1 (0·6)	17·6 (1·5)	16·5 (1·8)	6·6 (2·0)	2·3 (0·6)
Digoxigenin mono-digitoxoside	11·2 (0·7)	5·8 (1·2)	3·8 (0·5)	2·1 (0·6)	1·4 (0·3)	16·7 (0·8)	11·2 (2·0)	8·0 (0·1)	6·6 (2·1)	3·4 (0·4)	2·3 (0·3)	0·7 (0·2)	8·8 (3·1)	12·4 (0·6)	12·5 (1·0)	7·5 (1·6)	3·5 (1·0)	1·4 (0·5)
Digoxigenin	20·9 (0·9)	18·2 (4·1)	14·9 (1·5)	1·9 (0·9)	1·9 (0·4)	55·1 (2·6)	45·6 (6·4)	25·1 (1·4)	6·9 (1·8)	4·8 (0·5)	3·1 (0·7)	0·5 (0·4)	83·7 (5·2)	54·0 (1·2)	20·4 (1·7)	13·8 (0·6)	3·9 (0·7)	2·0 (1·4)

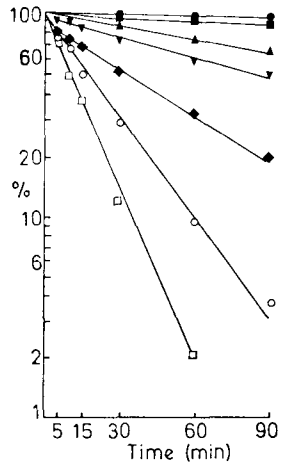

FIG. 1. Relation between percent digoxin remaining and duration of incubation with hydrochloric acid at 37° in a shaking water bath at various levels of pH, ●—3, ■—2·5, ▲—2·0, ▼—1·8, ◆—1·5, ○—1·2, □—0·9.

as pH was reduced below this level, the rate of hydrolysis showed a steady increase with increasing hydrogen ion concentration. For each pH studied, the amount of radioactivity remaining as digoxin decreased exponentially with time.

The rates of hydrolysis of digoxin incubated with hydrochloric acid or gastric juice at pH 1·2 and 37°, with and without agitation, are compared in Table 2. Hydrolysis without agitation was significantly faster with hydrochloric acid than with gastric juice, at all times studied ($P<0·05$). However, when agitation was used (Table 2), the differences were reversed or were less marked. This may be accounted for by the finding that agitation of hydrochloric acid had virtually no influence ($P>0·05$) on the rate of hydrolysis of digoxin, whereas agitation of gastric juice uniformly resulted in faster hydrolysis ($P<0·025$), both in terms of the rate of disappearance of digoxin and the appearance of digoxigenin and its mono- and bis-digitoxosides.

Table 2. *Comparison of hydrolysis of digoxin with hydrochloric acid and gastric juice at pH 1·2 and the influence of agitation.* Incubation was at 37°. Means with s.d. of 4 runs.

		% Radioactivity remaining as digoxin Incubation, min					
		5	10	15	30	60	90
Without agitation	Hydrochloric acid	76·2 (4·0)	63·4 (3·2)	54·3 (5·1)	29·1 (7·1)	14·2 (7·2)	4·6 (1·8)
	Gastric juice	82·3 (4·1)	72·3 (3·2)	63·6 (1·4)	45·4 (2·6)	20·5 (1·8)	10·1 (1·8)
Agitation	Hydrochloric acid	76·7 (3·7)	66·7 (9·5)	49·1 (2·7)	28·8 (5·8)	9·4 (2·7)	3·7 (1·0)
	Gastric juice	73·3 (2·1)	63·1 (1·8)	54·8 (2·5)	34·4 (2·6)	15·2 (1·1)	5·7 (3·0)

Temperatures influenced the rate of hydrolysis of digoxin. The rate, when digoxin was incubated with agitation in hydrochloric acid of pH 1·2 at 37° is shown in Table 1, and at 25° in Table 3. The rate was significantly slower at 25° at all times ($P<0·01$). For example at 25°, 84 and 57% remained as digoxin after 30 and 90 min respectively, compared with 29 and 3·7 when at 37°.

Table 3. *In vitro hydrolysis of [^{3}H]digoxin-12α incubated at 25° with hydrochloric acid pH 1·2 and agitation.* Percentage radioactivity in fractions eluted after t.l.c.*. Means and s.d. of 4 determinations.

	Duration of incubation					
	5 min %	10 min %	15 min %	30 min %	60 min %	90 min %
Digoxin	93·8 (0·9)	92·6 (1·9)	89·8 (0·3)	83·7 (1·0)	69·0 (1·1)	56·7 (2·1)
Digoxigenin bis-digitoxoside	3·6 (1·4)	4·4 (0·9)	5·5 (0·6)	7·9 (0·6)	12·6 (1·2)	16·7 (0·7)
Digoxigenin mono-digitoxoside	1·3 (0·3)	1·4 (0·5)	1·8 (0·5)	3·4 (0·4)	6·6 (0·3)	9·6 (0·9)
Digoxigenin	1·3 (0·3)	1·6 (0·8)	2·9 (0·7)	5·0 (0·9)	11·7 (0·4)	17·0 (1·5)

* Silica gel chromatograms were developed using chloroform–ethyl acetate–glacial acetic acid (25 : 25 : 1) as the solvent system.

A 0·25 mg digoxin tablet (Lanoxin Lot No. 401F) was incubated for various times at 37° in gastric juice of pH 1·2 and aliquots spotted on a chromatogram. The intensity of the spots under ultraviolet light was estimated visually. When incubation was carried out for 30 min, about 50% remained as digoxin compared with 37% when a solution of [^{3}H]digoxin-12α was used. The digoxin tablet appeared to have disintegrated completely within 5 min.

Preliminary *in vitro* studies indicate that acid hydrolysis of digitoxin compares with that of digoxin.

Gastric aspirates were taken serially from a volunteer who received [^{3}H]digoxin-12α via a nasogastric tube during a pentagastrin infusion. [^{3}H]Digoxin and products of hydrolysis were extracted from the aspirates and separated using DEAE Sephadex LH-20 column chromatography (Fig. 2 Table 4). The rate of hydrolysis was similar to that found in the *in vitro* study with hydrochloric acid at pH 0·9 (Table 1). By 40 min, 90% of the radioactivity was in the bis-digitoxoside,

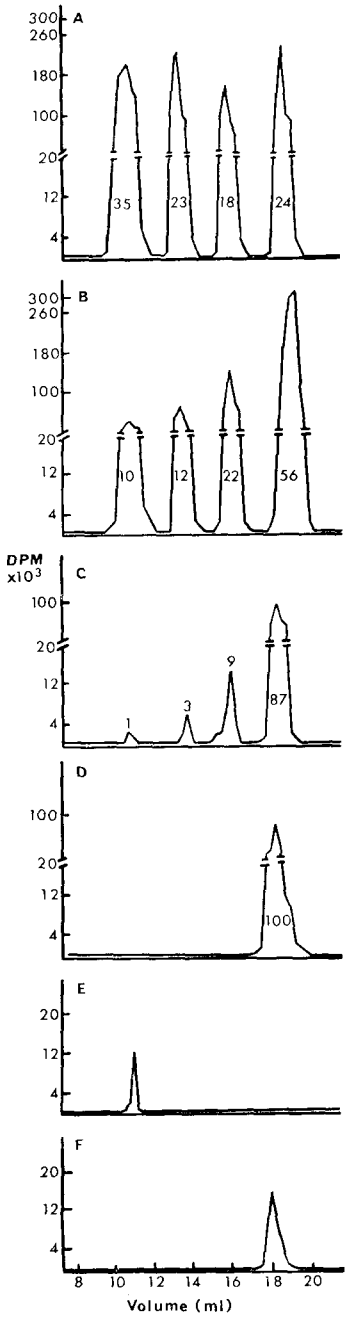

FIG. 2. DEAE Sephadex LH-20 elution profiles of the gastric aspirates obtained at A—20, B—40, C—60 and D—90 min after administration of 150 μCi [³H] digoxin-12α to a volunteer via a nasogastric tube during a pentagastrin infusion. The peaks in the 20 min specimen represent from left to right; digoxin, digoxigenin-bis-digitoxoside, digoxigenin-mono-digitoxoside, and digoxigenin. The lower two graphs show the elution profiles for E—digoxin and F—digoxigenin standards.

Table 4. *Intragastric hydrolysis of digoxin: percentage of total radioactivity in fractions eluted with DEAE Sephadex LH-20 column chromatography of gastric juice.*

Time* (min)	Digoxin %	Bis-digitoxoside %	Mono-digitoxoside %	Digoxigenin %
20	35·2	23·4	17·9	23·5
40	10·1	12·0	21·7	65·2
60	1·2	2·6	8·9	87·3
90	—	—	—	100·0

* After instillation of [³H]digoxin-12α into the stomach of a volunteer through a nasogastric tube.

mono-digitoxoside and digoxigenin peaks. By 60 min only 1% remained in the digoxin peak and by 90 min 100% of the radioactivity was in the digoxigenin peak, indicating complete hydrolysis of digoxin and the mono-and bis- digitoxosides of digoxigenin. The pH was 0·94 during pentagastrin infusion immediately before administration of the digoxin and was unchanged 90 min later. Total radioactivity recovered in the gastric aspirates was 30 μCi, or 20% of the dose given.

The elution profiles obtained from DEAE Sephadex LH-20 chromatography of chloroform–ethanol extracts of urine specimens obtained from the same volunteer during and serially for 5 days after the pentagastrin infusion, revealed that 100% of the radioactivity was under the digoxigenin peak in all specimens. The elution profiles obtained from urine voided 0–2 h and 24–36 h after ingestion of [³H]digoxin-12α are shown in Fig. 3. 22% of the [³H]digoxin-12α given was recovered over the 5 day period. In contrast, when the same volunteer received [³H]digoxin-12α orally without a pentagastrin infusion, chromatography elution profiles of urine samples showed that 92·6 and 95·5% of the radioactivity excreted was in the digoxin peak in the 0–2 h and the 24–36 h specimens respectively (Fig. 3); 53 % of the ingested radioactivity was recovered over the 5 day collection period.

DISCUSSION

Our results illustrate that intragastric hydrolysis of digoxin can occur at approximately the same rate as acid hydrolysis *in vitro*, for a given pH. Moreover, the observations that complete intragastric hydrolysis of [³H]digoxin-12α can occur in man within 60–90 min and that urinary excretion may be entirely as digoxigenin, could have clinical significance in certain patients when digoxin is given by the oral route. This significance could relate to dosage form, to dose as influenced by altered rates

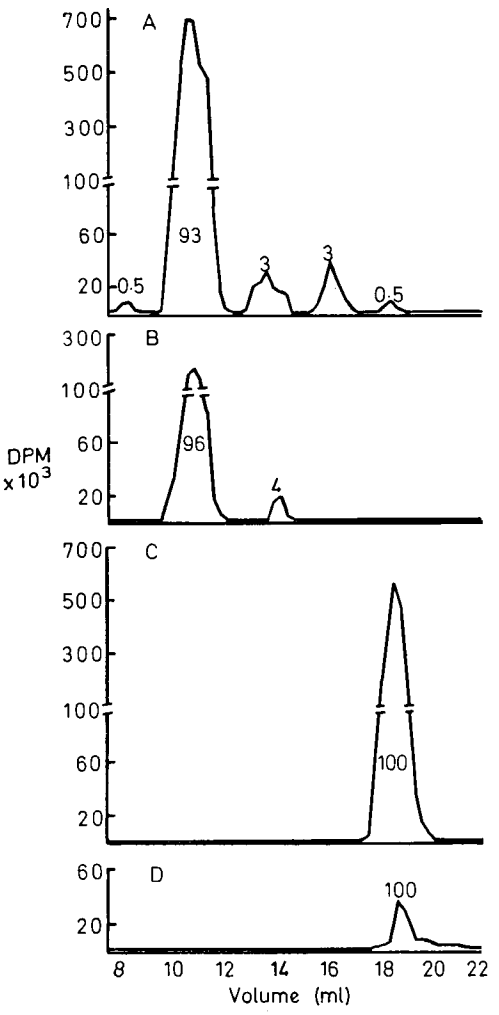

FIG. 3. DEAE Sephadex LH–20 elution profiles of extracts of urine voided by a volunteer 0–2 (A, C) and 24–36 h (B–D) after administration of [^{3}H]digoxin-12α. Profiles A and B were obtained without pentagastrin stimulation and profiles C and D after stimulation by pentagastrin when gastric pH was 0·94. Fractions eluted in the 0–2 h specimen (A) were from left to right; dihydrodigoxin, digoxin, digoxigenin-bis-digitoxoside, digoxigenin-mono-digitoxoside and digoxigenin. Only digoxigenin appeared in the urine when pentagastrin was infused.

of absorption and elimination and to reduced myocardial action.

Under abnormal circumstances, as with the Zollinger-Ellison Syndrome (Ellison & Wilson, 1964; Shay, Chey & others, 1962) gastric pH may fall to below 1. However, it is the normal lower range that will be the critical factor which determines how often appreciable intragastric hydrolysis occurs and this has not been well defined. Gastric pH may normally reach 1–2 (Fordtran & Sleisenger, 1973; Milton-Thompson, Jenkins & others, 1974) and therefore gastric hydrolysis may at times be clinically significant and could explain the results of Clark & Kalman (1974) who found 32 and 40% glycoside breakdown products in the urine of 2 patients but only 0–1% in 4 others. However, 13 subjects* and the subject of this study (Fig. 3A and B) excreted a maximum of 9% radioactivity as digoxigenin and its mono- and bis- digitoxosides in urine collected for 5 days or more after ingestion of [^{3}H]digoxin-12α. These results, and those of others (Beermann & others, 1972; Marcus, Burkhalter & others, 1966), who found no greater than 14% of ingested tritiated digoxin excreted as digoxigenin and its mono- and bis- digitoxosides, do not suggest that intragastric hydrolysis is ordinarily an important feature.

Beermann & others (1972) reported that during the first hour after [^{3}H]digoxin-12α was instilled into the stomach, approximately 10% of the label was absorbed by the stomach in 3 subjects and no uptake was observed in a fourth. However, values for pH ranged from 2·3–7·4. 40–60% of the label was taken up in all subjects in the upper small intestine. Only small amounts of digoxigenin and its mono- and bis-digitoxosides were excreted in the urine. In this present study, only digoxigenin appeared in urine after pentagastrin stimulation, in spite of the demonstration of digoxigenin and bis- and mono-digitoxoside up to 60 min in gastric fluid. This suggests either that there was no gastric emptying before hydrolysis was complete between 60 and 90 min or that digoxigenin was absorbed from the stomach with no absorption of digoxin and the mono- and bis-digitoxosides of digoxigenin. The latter explanation must be considered for only 20% of administered radioactivity was recovered in gastric aspirates in spite of the attempt to completely aspirate the stomach contents 90 min after drug administration. Only small volumes were intentionally aspirated at 0, 20, 40 and 60 min. 22% of the administered radioactivity was recovered in urine.

The demonstration in man and animals that digoxigenin and its mono- and bis-digitoxosides appeared in bile and in urine after [^{3}H]digoxin-12α was given intravenously (Abel, Luchi & others, 1965; Doherty, Flanigan & others, 1972; Klassen, 1974; Gault, Ahmed & others, 1976), and the narrow range found for these compounds in urine in our 13 subjects who ingested a solution of [^{3}H]

* Results in part published (Sugden, Ahmed & others, 1975).

digoxin-12α (Sugden, Ahmed & others, 1975), suggest that the glycosidic metabolites of digoxin may ordinarily be in part, or entirely formed after absorption, rather than being the result of intragastric hydrolysis. In addition, Beermann & others (1972) found no increase in urinary excretion of digoxigenin and its bis- and mono- digitoxosides when an aqueous solution of [^{3}H]digoxin-12α was ingested, compared with the results obtained following instillation directly into the jejunum.

There appears to be no information obtained directly in man on the relative inotropic effects of digoxigenin and its mono- and bis- digitoxosides compared with digoxin (Marcus, Ryan & Stafford, 1975). In cats, Bottcher, Lullmann & Proppe (1973), using a heart-lung preparation, found that at 50% of peak inotropic effect, the relative potency of the bis- and mono- digitoxosides of digoxigenin were 1·26 and 1·44, compared with digoxin taken as unity. Marcus & others (1975), recalculated the data of Henderson & Chen (1962) and Chen (1970) obtained in cats. On the basis of pmol mg^{-1} body weight, the relative toxicities, compared with digoxin as unity, were digoxigenin-bis-digitoxoside 1·0, digoxigenin-mono-digitoxoside 1·4 and digoxigenin 0·3. The ability to displace [^{3}H]ouabain from Na-K-ATPase has been reported (Marcus & others, 1975) to be greatest for digoxigenin-mono-digitoxoside, and to decrease in the following order: digoxigenin-bis-digitoxoside, digoxin and digoxigenin. Should all the digoxin be hydrolysed to digoxigenin as occurred in this study after pentagastrin stimulation, there would be appreciably less cardiac effect, and a lower serum digoxin concentration for a given dose of digoxin since digoxigenin has less affinity for digoxin specific anti-serum than digoxin (Marcus & others, 1975). Also the rate of disappearance from blood might be more rapid, as the half-life of digoxigenin has been reported to be only 3 h (Beermann & others, 1972), compared with an average of 1·3–1·4 days for digoxin (Doherty, Flanigan & Dalrymple, 1972; Brown & Abraham 1973) in those with normal renal function.

Clearly, considerable additional investigation will be required to evaluate the possible clinical significance of intragastric hydrolysis of cardiac glycosides.

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