Br. J. Pharmac. (1971), **41**, 661–673.

Metabolism of cardiac glycosides studied in the isolated perfused guinea-pig liver

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Summary

1. Metabolic degradation of tritiated ouabain, digoxin, and digitoxin has been investigated quantitatively using the isolated perfused guinea-pig liver. The cardiac glycosides and their metabolites have been extracted from the plasma, liver, and bile by different solvents and identified as far as possible by radiochromatographic analysis.

2. The total metabolic activity in the experimental system was localized in the liver.

3. The hydrophilic glycoside ouabain could not penetrate into the metabolically active compartment of the liver and was, therefore, not degraded. The more lipophilic compound digitoxin, however, was completely degraded due to its high affinity for the metabolically active sites. The unchanged digitoxin cannot enter the aqueous bile fluid in contrast to its more hydrophilic metabolites.

4. The only detectable metabolic degradation of digoxin was a conjugation with glucuronic and/or sulphuric acid, but a cleavage of sugar molecules seemed not to occur.

5. In the case of digitoxin the metabolic processes are more complicated: sugar cleavage, conjugation, and C-12 hydroxylation take place simultaneously. An immediate hydroxylation of digitoxin leading to digoxin was not observed. After administration of digitoxin conjugation products as well as digoxigenin-bis-and digoxigenin-mono-digitoxosides were present in each of the compartments investigated, but the digitoxosides of digitoxigenin were intermediates in concentrations too low to be determined indicating a very high rate of conjugation and/or C-12 hydroxylation as compared with the cleavage of the digitoxoses.

6. A scheme for the metabolic pathways of the cardiac glycosides based on experimental results is presented. The metabolic behaviour of each of the three compounds involved is closely related to their physicochemical properties, especially the lipid solubility.

Introduction

In spite of fundamental contributions by Repke and co-workers (Repke, 1958, 1959a, b, c, d; Hermann & Repke, 1964a, b, c) and other investigators (Okita, Talso, Curry, Smith & Geiling, 1955a; Okita & Curry, 1955b; Katzung & Meyers, 1965; Brown, Ranger & Wright, 1955; Abel, Luchi, Peskin, Conn & Miller, 1965; Lage & Spratt, 1965, 1968; Marcus, Kapadia & Kapadia,

1964 ; Marcus, Burkhalter, Cuccia, Paulovich & Kapadia, 1966 ; Markus, Paulovich, Burkhalter & Cuccia, 1967) the metabolism of cardiac glycosides remains, in several respects, subject to debate.

For digoxin and digitoxin the sequence of metabolic degradation seems to be well established. Experiments using liver slices have demonstrated a stepwise breakdown of the tridigitoxosides into more lipophilic and subsequently hydrophilic metabolic products by splitting off the sugar moieties and coupling of the genin and/or the epi-genin with glucuronic or sulphuric acid (Hermann & Repke, 1964a). Moreover, digitoxin may be hydroxylated at the C-12 atom (Repke, 1963, 1966 ; Wilson, 1969).

There is little information concerning the quantitative relationship between lipophilic and hydrophilic metabolites formed in the organism and their distribution in blood and tissues (Förster, Grade & Schulzek, 1967 ; Gonzales & Layne, 1960). Most of the earlier work is limited to the investigation of lipophilic metabolites in excretory products (Repke, 1959c, 1959d ; Brown *et al.*, 1955 ; Ashley, Brown, Okita & Wright, 1958 ; Marcus *et al.*, 1964, 1966, 1967 ; Wright, 1960), since only a few investigators have also taken note of the chloroform insoluble hydrophilic metabolites (Okita *et al.*, 1955a,b ; Katzung & Meyers, 1965 ; Abel *et al.*, 1965). The commonly used methods of extraction with chloroform necessarily direct the attention to lipophilic metabolites so that the polar metabolites are neglected (Griffin & Burstein, 1967). Using the extraction method described below, it is possible to determine the amounts of unchanged drug together with its polar and its lipid soluble metabolites in a single extract.

Furthermore little is known about the fate of the metabolic degradation products in the organism. This is particularly true for the binding of metabolites of the cardiac glycosides to proteins in the blood and to other tissues. In a previous paper the kinetic aspects of plasma elimination, uptake by the liver, and biliary excretion of ouabain, digoxin, and digitoxin have been described and the influence of metabolic degradation upon these processes discussed (Kolenda, Lüllmann, Peters & Seiler, 1971).

In this paper, quantitative results are given concerning the degradation of the three glycosides and the influence of this degradation upon plasma protein binding. The findings emphasize the close correlation between physicochemical properties and rate of metabolic degradation of the cardiac glycosides.

Methods

A detailed description of a rapid dissection method, the technique of perfusion, and some tests of function have been given in earlier papers (Berg, Kolenda, Peters & Seiler, 1970 ; Kolenda *et al.*, 1971). The blood obtained from guinea-pigs was treated with 5,000 I.U. of heparin/100 ml and 20 mg streptomycin/100 ml and diluted with Tyrode solution 2:1. The perfusion flow was maintained at approximately (0·7 ml/g liver)/min throughout the experiment.

Methods of extraction

At the end of the perfusion experiments the radioactive compounds were extracted from plasma, bile and liver. Plasma was obtained by centrifuging the perfusion fluid twice. Plasma (150 ml) was mixed with three times the volume of absolute

alcohol for 30 min and the precipitated protein obtained was separated by centrifugation. The clear supernatant was filtered and evaporated to dryness at reduced pressure. The residue thus obtained was taken up in 20 ml 70% alcohol and filtered. The filtrate was purified three times with petroleum ether (boiling range 50–70° C) and once more evaporated to dryness. The residue was taken up in 5 ml chloroform/methanol 1:1, filtered, and the clear extract thus obtained was used for chromatographic analysis.

The bile secreted during the perfusion (average volume 6–8 ml) was treated in exactly the same way as described for plasma.

The liver (average weight 25 g) was homogenized by means of an Ultra-Turrax homogenizer in four times its volume of absolute ethanol and was continuously stirred for half an hour. Subsequent treatment of the homogenate was the same as for plasma and bile. The volume of the final chloroform/methanol extract was also 5 ml. Recovery of radioactivity by the extraction procedure was 60% for plasma and liver and 80% for bile. This loss of radioactivity is caused by several necessary filtration and purification steps. Water and lipid soluble compounds are unspecifically adsorbed to the same extent as shown by recovery experiments.

Chromatographic analysis

Ten to fifty microlitres of the extracts from plasma, liver, and bile were spotted with a micropipette on silica-G-thinlayer-plates (silica G according to Stahl, 1967), 0·2 mm layer. Two different solvents were used: cyclohexane/acetone/acetic acid 49:49:2 to separate the apolar metabolites and chloroform/methanol/H_2O 65:30:5 to separate the polar compounds like ouabain, metabolites, and digitoxose. The flow distance was 13 cm. The radioactivity of the chromatogram was monitored by means of a radio scanner (Packard, model 7201). To identify the labelled substances the R_F values of a series of unlabelled glycosides and possible metabolites were determined. The unlabelled compounds were made visible by a colour reaction using anisaldehydesulphuricacid reagent (Stahl, 1967).

Determination of plasma protein binding

The plasma protein binding of ouabain, digoxin and digitoxin and the corresponding metabolites was estimated by means of ultracentrifugation (Scholtan, Schlossmann & Rosenkranz, 1966). At definite intervals throughout the perfusion experiments samples of 15 ml volume each were taken from the perfusion medium. Six and a half millilitres of the subsequently obtained plasma were centrifuged in cellulose nitrate tubes (diameter 1 cm) for 15 h at 40,000 *g* and 20° C in a Beckman ultracentrifuge (type 50 rotor). The test tubes were frozen at −30° C. For the analysis of the non-protein bound radioactivity, a sample was taken from a layer at least 0·5 cm below the surface to avoid contamination by the lipoid-protein containing surface layer. The concentration of the total radioactivity in plasma was determined before ultracentrifugation.

The plasma protein concentration in the perfusion medium determined by the Biuret-method amounted to 30–40 mg/ml. No protein could be detected in the layer which was used for the determination of concentration of free radioactivity.

Measurement of radioactivity

Samples of plasma and bile, of the extracts of plasma, liver, and bile and of the protein-free plasma were counted in a liquid scintillation counter (Packard). For details of the methods see Kolenda *et al.* (1971).

Compounds and reagents

Tritiated ouabain, digoxin, and digitoxin were obtained from New England Nuclear Corp. The compounds were randomly labelled and had the following specific activities: ouabain 20 mCi/mg; digoxin 11·5 mCi/mg; digitoxin 7·5 mCi/mg. The final concentrations of the administered glycosides were $3{\cdot}4 \times 10^{-8}$M ouabain, 9×10^{-8}M digoxin, and $1{\cdot}4 \times 10^{-7}$M digitoxin. The following substances, supplied by Boehringer-Mannheim were used for the chromatographic analysis and identification of the metabolites: digitoxin, digitoxigenin, digitoxigenin-monodigitoxoside, digitoxigenin-bisdigitoxoside, digoxin, digoxigenin, digoxigenin-monodigitoxoside, digoxigenin-bisdigitoxoside and digitoxose.

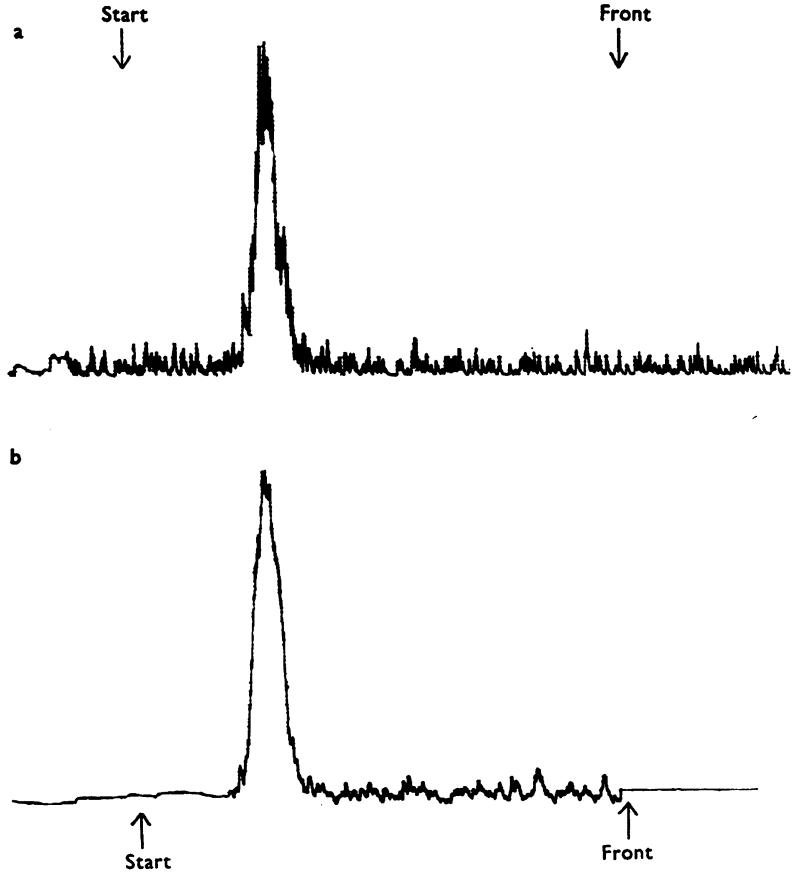

FIG. 1. Perfusion of the isolated guinea-pig liver by guinea-pig blood. Analysis by means of radio thin layer chromatography of the extracts from the plasma after administration of ^{3}H-ouabain. (a) Control experiment: the liver was not included in the circulation system; circulation period, 3 hours. (b) The liver was perfused by a medium containing ^{3}H-ouabain; circulation period, 3 hours. Solvent: cyclohexane/acetone/acetic acid (49:49:2).

Results

At the end of the 3 h perfusion experiments the overall radioactivity was distributed as follows: ouabain was not excreted via the liver but was distributed between plasma and liver; the latter contained approximately 40% of the total activity. After administration of digoxin or digitoxin the greater part of the radioactivity was found in the bile fluid. About 20% remained in the plasma and approximately 10% was found in the liver.

Radiochromatographic investigations

As shown in Fig. 1, only unchanged ouabain could be extracted from the plasma and liver after perfusion for 3 hours. No metabolites could be detected with either of the two solvents used.

Figures 2 and 3 show the findings after administration of digoxin. To see if a degradation of the apolar glycosides occurred in the blood, control experiments were

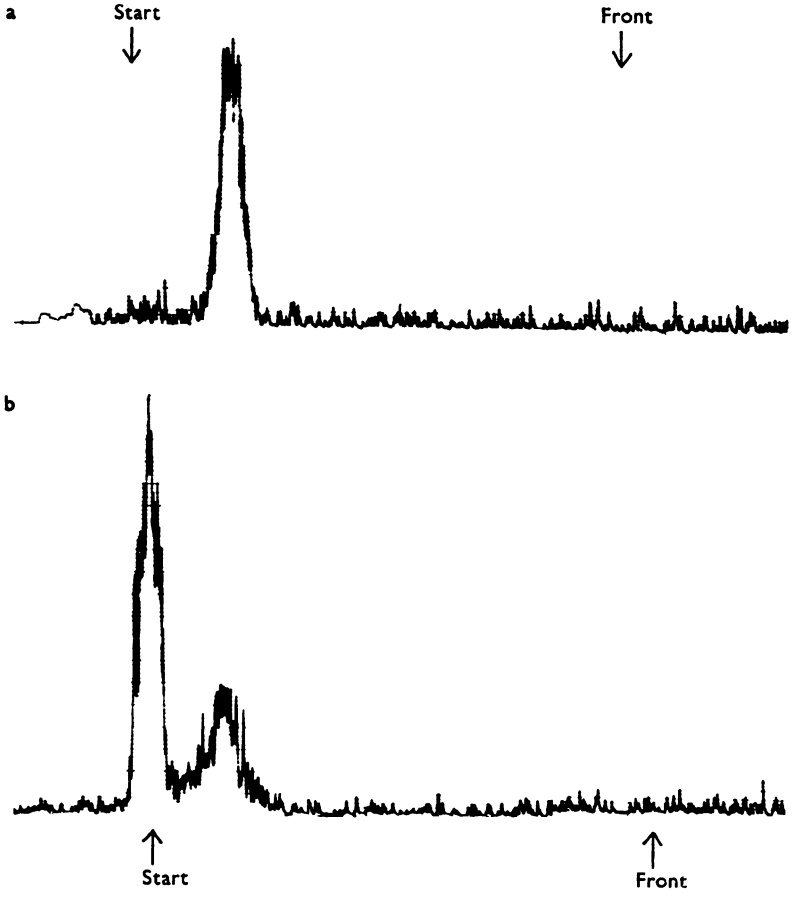

FIG. 2. Perfusion of the isolated guinea-pig liver by guinea-pig blood. Analysis by means of radio thin layer chromatography of the extracts from the plasma after administration of ^{3}H-digoxin. (a) Control experiment: the liver was not included in the circulation system; circulation period, 3 hours. (b) The liver was perfused by a medium containing ^{3}H-digoxin; circulation period, 3 hours. Solvent: cyclohexane/acetone/acetic acid (49:49:2).

carried out in which the liver was excluded from the circulation. For this purpose the portal and vena caval catheters were directly connected with each other, all other experimental conditions remaining identical. In these control experiments, digoxin could be extracted entirely unchanged from the plasma, indicating a complete lack of metabolic degradation (Fig. 2, top). The perfusion of the liver, however, yielded a completely different picture. An important peak was found at the start of the chromatogram. The low R_F value indicates that the radioactive material represented by this peak must be highly polar. This initial peak was followed by a second maximum with an R_F value corresponding to that of digoxin (Fig. 2, bottom).

The greater part of the radioactive material in the liver extract consisted of unchanged digoxin, while in the bile, a high polar peak appeared at the start preceding that of unmetabolized digoxin (Fig. 3).

After administration of digitoxin no degradation products were extracted in control experiments (Fig. 4, top), whereas in the liver perfusion experiments digitoxin

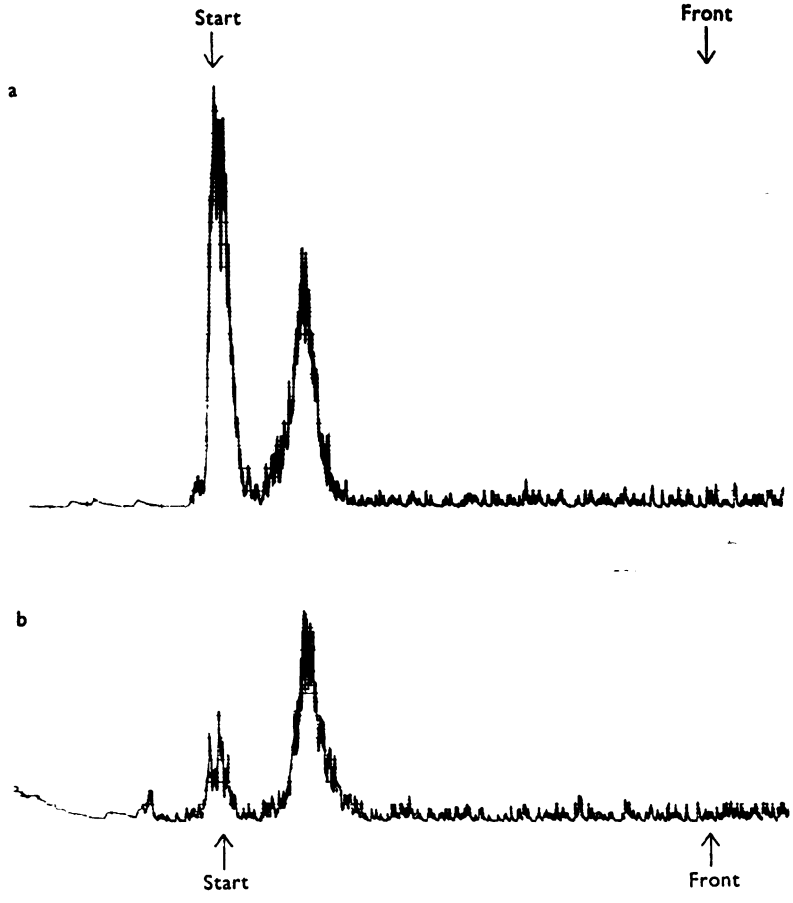

FIG. 3. Perfusion of the isolated guinea-pig liver by guinea-pig blood. Analysis by radio thin layer chromatography of the extracts from the bile (a) and the liver (b) after administration of ^{3}H-digoxin. Solvent: cyclohexane/acetone/acetic acid (49:49:2).

could no longer be detected in the plasma (Fig. 4, bottom). A high peak remained at the start of the chromatogram, followed by a smaller, less polar one, which on the basis of its R_F value probably represents the mono- and bis-digitoxosides of digoxigenin.

In the liver, mainly the mono- and bis-digitoxosides of digoxigenin were found, whereas in the bile fluid the polar peak at the start predominated (Fig. 5). Using the polar solvent the peaks situated at the starting point could be separated into several peaks not yet identified.

Digitoxose could be separated from the polar and the less polar metabolites by means of the polar solvent. The sugar moiety could only be detected in liver extract but not in extracts from plasma and bile.

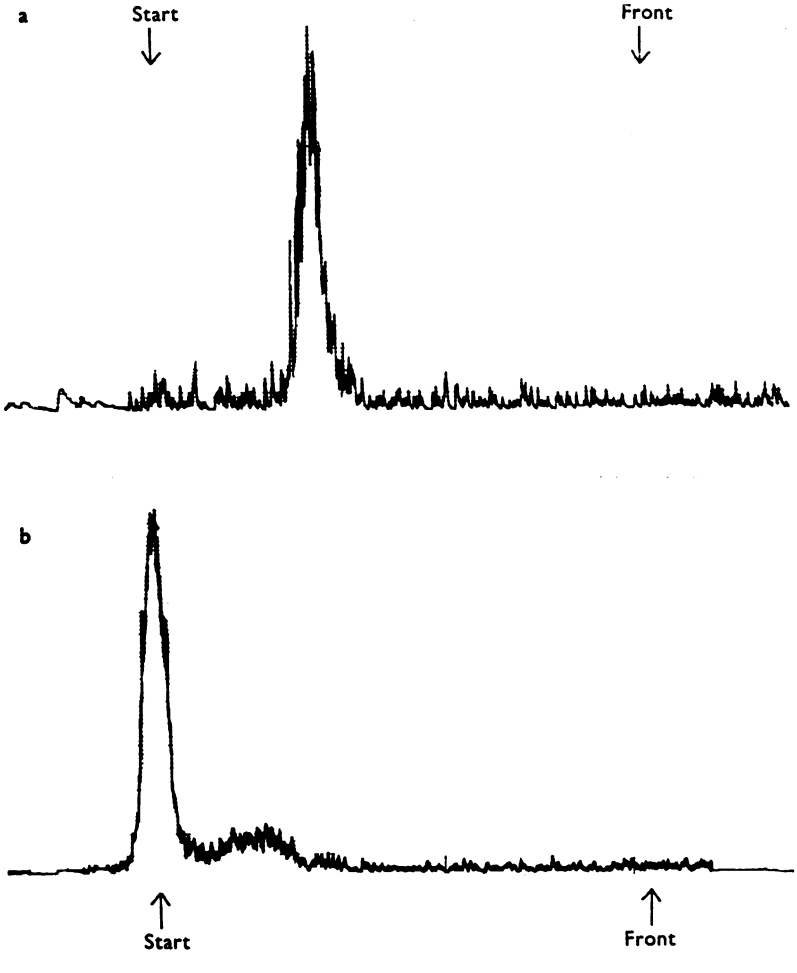

FIG. 4. Perfusion of the isolated guinea-pig liver by guinea-pig blood. Analysis by means of radio thin layer chromatography of the extract from the plasma after administration of ^{3}H-digitoxin. (a) Control experiment: the liver was not included in the circulation system circulation period, 3 hours. (b) The liver was perfused by a medium containing ^{3}H-digitoxin; circulation period, 3 hours. Solvent: cyclohexane/acetone/acetic acid (49:49:2).

Plasma protein binding

During the course of the perfusion experiment the non-protein bound portion of radioactive material after administration of ouabain remained constant around 95%. After administration of digitoxin, however, the non-protein-bound material significantly increased from approximately 10% to about 60% within one hour of perfusion and was maintained at this level (see Fig. 6). Plasma elimination, biliary excretion and metabolic degradation of digoxin took place, however, without any noticeable change in plasma protein binding of the radioactive compounds.

Discussion

The aim of this work was to investigate the metabolic degradation of some of the therapeutically important cardiac glycosides. The isolated perfused guinea-pig liver was chosen for the following reasons: (1) to avoid any pharmacokinetic interference with the circulation, tissue distribution, renal excretion and so on; (2) the

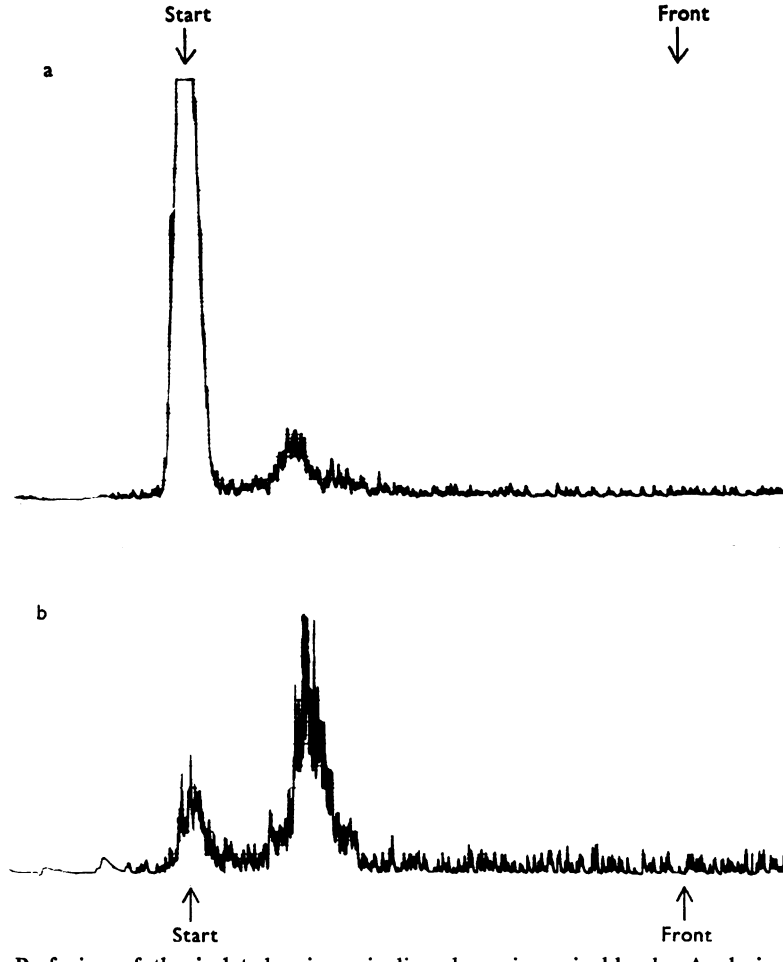

FIG. 5. Perfusion of the isolated guinea-pig liver by guinea-pig blood. Analysis by means of radio thin layer chromatography of the extracts from the bile (a) and the liver (b) after administration of ^{3}H-digitoxin. Solvent: cyclohexane/acetone/actic acid (49:49:2).

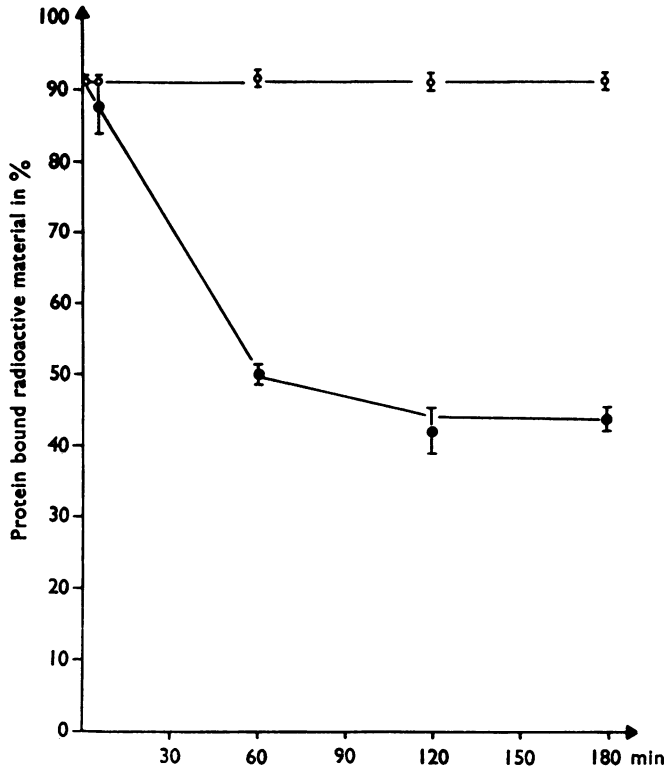

FIG. 6. Plasma protein binding of radioactive material in the course of the perfusion experiments with ^{3}H-digitoxin. The protein bound fraction decreases according to the metabolic degradation of digitoxin. Ordinate: protein bound radioactive material (%). Abscissa: time in minutes after administration of ^{3}H-digitoxin to the perfusion fluid. ○——○, Control experiments, in which the liver was not included in the artificial circulation. ●——●, Perfusion experiments. (Means ± S.E.M. n=6–8).

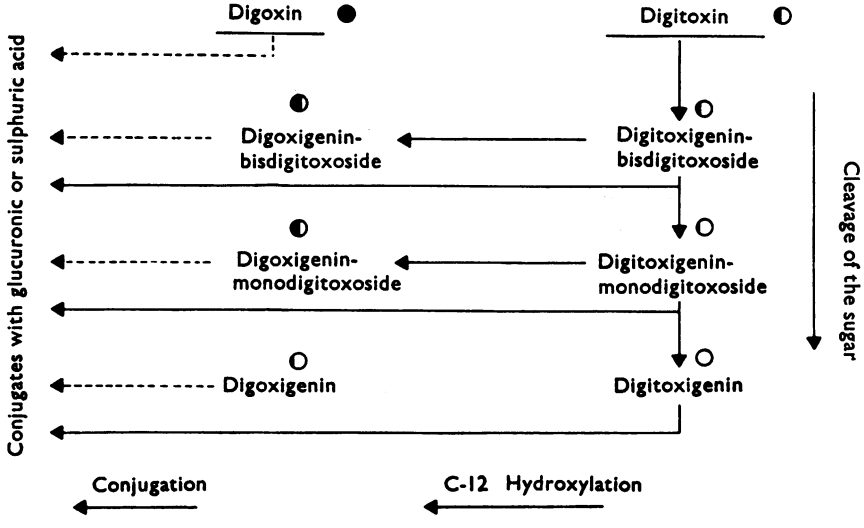

FIG. 7. Schematic presentation of the metabolic degradation of digoxin and digitoxin by the isolated perfused guinea-pig liver. - - - - →, Low rate of conjugation. ——→, High rate of conjugation, C-12 hydroxylation or sugar cleavage. ○, Highest lipid solubility. ●, Lowest lipid solubility of the compounds involved.

liver is the most important organ for the biotransformation ; and (3) the guinea-pig is the animal species that resembles humans most closely as far as the pharmacodynamic behaviour of the cardiac glycosides is concerned.

The method of extraction used in these experiments yields satisfactory quantitative results as compared with other methods (Wong & Spratt, 1963 ; Gonzales & Layne, 1960 ; Grimmer, Küssner & Lingner, 1960 ; Seipel, Hueber, Deutsch, Letz, Wichtl & Jentzsch, 1968), since originally applied glycoside, lipophilic and hydrophilic metabolites could be detected simultaneously.

In control experiments in which the liver was not included in the circulation system no metabolism of the three glycosides could be detected. This proves that the blood containing perfusion medium as such does not provoke metabolic degradation of the drugs involved. This finding confirms the observations by Hermann & Repke (1964c) and by Lüllmann, Peters & van Zwieten (1969) but differs from the results reported by Wright (1960). In our system, the degradation reactions can be localized in the liver only.

As stated under **Results** the plasma protein binding of ouabain as well as that of digoxin and its metabolites did not change in the course of the perfusion experiments. On the other hand the non-protein bound fraction of labelled compounds after administration of digitoxin increased from 10 to about 60%. This finding reflects the occurrence of newly formed, less lipophilic metabolites with lower affinity for plasma proteins since introduction of polar groups into drug molecules causes a decrease of the plasma protein binding (Scholtan, 1968 ; Kuschinsky, 1969).

The most simple situation was encountered in the case of ouabain which is neither metabolized nor excreted with the bile. This agrees with investigations in humans, where a metabolic alteration of the ouabain molecule has not yet been observed (Doherty, 1968 ; Marks, Dutta, Gauthier & Elliot, 1964).

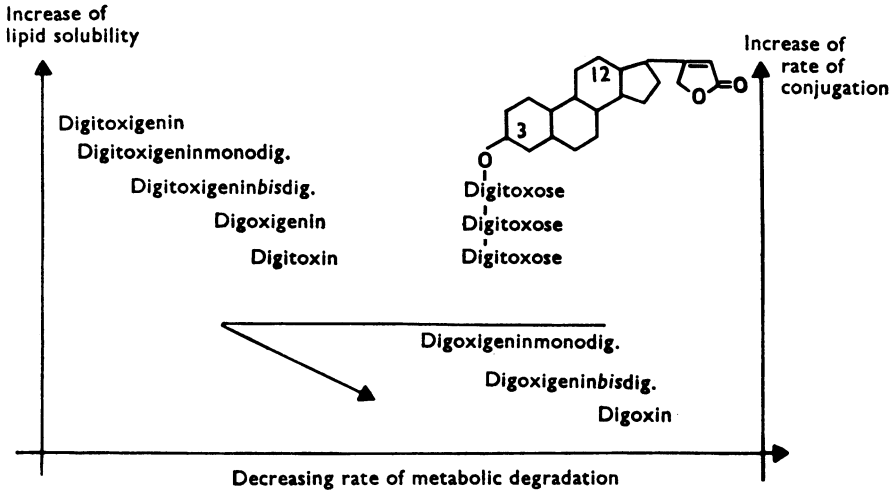

FIG. 8. Correlation between lipid solubility, rate of metabolic degradation, and rate of conjugation as obtained by experiments using the isolated perfused guinea-pig liver. The arrow marks a break in the order of the compounds listed since the little lipid soluble drugs below can be excreted with the bile in the unchanged form and moreover a sugar cleavage seems to be impossible.

On the other hand digitoxin is degraded completely within a short period of perfusion. This holds true even for concentrations as high as 4 mg digitoxin per 250 ml of perfusion fluid. Three hours after the addition of this amount no original glycoside could be detected any more. However, several metabolites could be demonstrated in plasma, liver tissue and bile. Surprisingly, the less polar metabolites that could be recovered were C-12 hydroxylated products, which contained the digoxigenin ring moiety. The hydrophilic metabolites which occurred simultaneously probably consisted of steroids conjugated with either glucuronic or sulphuric acid (Hermann & Repke, 1964a). The situation after administration of digitoxin has been represented schematically in Fig. 7. Most of the conjugation products contained the digitoxigenin ring moiety. This rapid metabolism explains why chiefly water soluble products are excreted with the urine not only in intact guinea-pigs (Gadke & van Zwieten, 1969) and rabbits (Griffin & Burstein, 1967) but also in humans (Doherty, 1968 ; Okita, 1964 ; Okita & Curry, 1955b) after administration of digitoxin.

Digoxin was degraded only in part and could be detected in the unchanged form in plasma, liver tissue, and bile. In contrast to digitoxin the original glycoside molecule may itself be conjugated. Since the degradation rate of digoxin in the human liver seems to be lower than in guinea-pig liver (Ruiz-Torres, 1970, Hermann & Repke, 1964c) it follows that unchanged digoxin is the major excretion product in humans (Doherty, 1968).

In Fig. 7 the probable pathways for the metabolism of the digitalis glycosides based upon our results have been summarized. The different steps elucidated in detail are as follows: a cleavage of sugar molecules from digoxin does not occur since neither digoxigenin-mono-digitoxoside nor digoxigenin-bis-digitoxoside could be demonstrated in any of the compartments. The rate of conjugation of digoxigenin-bis-digitoxoside and digoxigenin-mono-digitoxoside is so slow that these two compounds should accumulate as shown in the case of digitoxin. Digoxin itself will be conjugated at a rather low rate, since the unchanged glycoside is present after longer periods of time ; even within 4 h of perfusion one-third of the digoxin originally added is still present in the unchanged form. The coupling products of digoxin consist of one or two polar compounds which could be detected in the bile and plasma. Free digitoxose molecules could not be demonstrated.

Digitoxin itself cannot be hydroxylated at C-12, since digoxin, the C-12 hydroxylation product of digitoxin is not formed. However, the C-12 hydroxylation gains importance at the level of digitoxosides and this particular process determined the rate of the cleavage of the sugar moieties, resulting in an accumulation of digoxigenin-bis-digitoxoside and digoxigenin-mono-digitoxoside. However, the concentrations of digitoxigenin-bis-digitoxoside and digitoxigenin-mono-digitoxoside are so low that these two intermediates are not detectable. The main pathway for the breakdown of digitoxin is represented by a direct conjugation of the two digitoxosides and the genin. For the latter compounds the rate of conjugation was higher than the rate of hydroxylation. The group of conjugation products of digitoxin metabolism consists of several polar compounds. Hydrolysis of these compounds yields chiefly digitoxigenin and a smaller portion of digoxigenin, indicating a direct coupling of the digitoxosides and the genin (or possibly epigenin: Hermann & Repke, 1964a). Digitoxose can be traced in the liver tissue after administration of digitoxin. This finding emphasizes the importance of the cleavage of the sugar moieties ; indeed,

Domschke, Meinecke & Domagk (1969) have recently demonstrated the presence of a digitoxose dehydrogenase in the rat liver.

The relative importance of the different metabolic pathways (conjugation, hydroxylation and sugar cleavage) may be dependent upon the different physicochemical properties of the compounds involved. In Fig. 8 the digitalis glycosides and their derivatives have been arranged according to their lipid solubility and rate of metabolic degradation. This figure clearly demonstrates a correlation between the lipophilic nature and the rate of metabolic degradation. The more polar compounds digoxin, digoxigenin-bis-digitoxoside and digoxigenin-mono-digitoxoside represent a group which is characterized by a complete lack of cleavage of the digitoxose molecules. On the other hand the ability of these compounds to invade the bile fluid is obvious. Furthermore, the rate of conjugation is low in this group as compared with the compounds with higher lipid solubility. The more lipid soluble derivatives from digitoxin are metabolized at an increasing rate. Accordingly their concentration in our experimental system remains so low that they cannot be traced. Their transient existence can only be proved by the identification of their conjugates. In experiments with liver slices (Lauterbach & Repke, 1960) the intermediates just mentioned accumulate because of the lower capability of conjugation of liver slices.

The significance of the reported results and the metabolic scheme for the pharmacokinetic behaviour of the cardiac glycosides used therapeutically in patients can only be discussed to a limited extent. The comparably short half life of digoxin is due to the fact that it is excreted in the unchanged form and that the conjugation rate is relatively slow. Digitoxin cannot be excreted and is metabolized rapidly, the main metabolic products will not be eliminated either.

The authors are grateful to Mrs. Ulrike Kaiser for skilful technical assistance.

REFERENCES

ABEL, R. M., LUCHI, R. J., PESKIN, G. M., CONN, H. L. & MILLER, L. D. (1965). Metabolism of digoxin: role of the liver in tritiated digoxin degradation. *J. Pharmac. exp. Ther.*, **150**, 463–468.

ASHLEY, J. J., BROWN, B. T., OKITA, G. T. & WRIGHT, S. E. (1958). The metabolites of cardiac glycosides in human urine. *J. biol. Chem.*, **232**, 315.

BERG, J. W., KOLENDA, K. D., PETERS, T. & SEILER, K. U. (1970). Methodik der Perfusion isolierter Meerschweinchenlebern. *Ärztliche Forschung*, **8**, 240–244.

BROWN, B. T., RANGER, D. & WRIGHT, S. E. (1955). The excretory products of lanatoside C and digitoxin in the rat. *J. Pharmac. exp. Ther.*, **113**, 353–358.

DOHERTY, J. E. (1968). The clinical pharmacology of digitalis glycosides: a review. *Am. J. med. Sci.*, **255**, 382–414.

DOMSCHKE, W., MEINECKE, O. & DOMAGK, G. F. (1969). Untersuchungen zum Stoffwechsel des Digitaliszuckers Digitoxose in der Ratte. *Arch. exp. Path. Pharmak.*, **265**, 149–155.

FÖRSTER, W., GRADE, K. & SCHULZEK, S. (1967). The uptake and content of digitoxin and its metabolites in the heart muscle of rats and guinea-pigs after acute and chronic application. *Biochem. Pharmac.*, **16**, 1165–1174.

GADKE, J. & VAN ZWIETEN, P. A. (1969). Vergleichende Untersuchungen am Meerschweinchen über Serumaktivität, Ausscheidung und Gewebsverteilung von ^{3}H-Digitoxin und ^{3}H-Digitoxigenin. *Arch. exp. Path. Pharmak.*, **264**, 234.

GONZALES, L. F. & LAYNE, E. C. (1960). Studies on tritium-labelled digoxin. Tissue, blood and urine determinations. *J. clin. Invest.*, **39**, 1578–1583.

GRIFFIN, C. L. & BURSTEIN, S. H. (1967). Metabolism of cardiac glycosides. *Biochem. Pharmac.*, **16**, 447–454.

GRIMMER, G., KUSSNER, K. & LINGNER, K. (1960). Resorption und Abbau herzwirksamer Glykoside: 1. Mitteilung: Isolierung, Identifizierung und quantitative Bestimmung kleiner Mengen von Cardenolid-Glykosiden aus tierischen Materialien. *Arzneimittel-Forsch.*, **10**, 29.

HERMANN, I. & REPKE, K. (1964a). Konjugation von Cardenolidgeninen mit Schwefelsäure und Glucuronsäure. *Arch. exp. Path. Pharmak.*, **248**, 370–386.

HERMANN, I. & REPKE, K. (1964b). Über Speciesunterschiede in der Biotransformation von Digitoxin. *Arch. exp. Path. Pharmak.*, **247**, 35–48.

HERMANN, I. & REPKE, K. (1964c). Entgiftungsgeschwindigkeit und Kumulation von Digitoxin bei verschiedenen Species. *Arch. exp. Path. Pharmak.*, **247**, 19–34.

KATZUNG, B. G. & MEYERS, F. H. (1965). Excretion of radioactive digitoxin by the dog. *J. Pharmac. exp. Ther.*, **149**, 257–262.

KATZUNG, B. G. & MEYERS, F. H. (1966). Biotransformation of digitoxin in the dog. *J. Pharmac. exp. Ther.*, **154**, 575–580.

KOLENDA, K.-D., LÜLLMANN, H., PETERS, T. & SEILER, K.-U. (1971). Plasma concentration, uptake by the liver, and biliary excretion of tritiated cardiac glycosides studied by means of the isolated perfused guinea-pig liver. *Br. J. Pharmac.*, **41**, 648–660.

KUSCHINSKY, K. (1969). Über die Bindungseigenschaften von Plasmaproteinen für Herzglykoside. *Arch. exp. Path. Pharmak.*, **262**, 388–398.

LAGE, G. L. & SPRATT, J. L. (1965). H^3-digoxin metabolism by adult male rat tissues *in vitro*. *J. Pharmac. exp. Ther.*, **149**, 248–256.

LAGE, G. L. & SPRATT, J. L. (1968). Species and sex variation in the hepatic metabolism of H^3-digoxin *in vitro*. *J. Pharmac. exp. Ther.*, **159**, 182–193.

LAUTERBACH, F. & REPKE, K. (1960). Die fermentative Abspaltung von D-Digitoxose, D-Cymarose und L-Thevetose aus Herzglykosiden durch Leberschnitte. *Arch. exp. Path. Pharmak.*, **293**, 196–218.

LÜLLMANN, H., PETERS, T. & VAN ZWIETEN, P. A. (1969). The distribution of ^{3}H-labelled cardenolides between isolated guinea-pig atrial tissue and circulating oxygenated whole blood. *Br. J. Pharmac.*, **36**, 276–285.

MARCUS, F. J., KAPADIA, G. J. & KAPADIA, G. G. (1964). The metabolism of digoxin in normal subjects. *J. Pharmac. exp. Ther.*, **145**, 203–209.

MARCUS, F. J., BURKHALTER, L., CUCCIA, C., PAVLOVICH, J. & KAPADIA, G. G. (1966). Administration of tritiated digoxin with and without a loading dose. *Circulation*, **34**, 865–874.

MARCUS, F. J., PAVLOVICH, J., BURKHALTER, L. & CUCCIA, C. (1967). The metabolic fate of tritiated digoxin in the dog: a comparison of digitalis administration with and without a loading dose. *J. Pharmac. exp. Ther.*, **156**, 548–556.

MARKS, B. H., DUTTA, S., GAUTHIER, J. & ELLIOT, E. (1964). Distribution in plasma uptake by the heart, and excretion of ouabain-^{3}H in human subjects. *J. Pharmac. exp. Ther.*, **145**, 351–356.

OKITA, G. T., TALSO, P. J., CURRY, J. H., SMITH, F. D. & GEILING, E. M. K. (1955a). Metabolic fate of radioactive digitoxin in human subjects. *J. Pharmac. exp. Ther.*, **115**, 371–379.

OKITA, G. T. & CURRY, J. H. (1955b). Biotransformation of radioactive digitoxin in humans. *Fedn Proc.*, **18**, 429.

OKITA, G. T. (1964). Metabolism of radioactive glycosides. *Pharmacologist*, **6**, 45.

REPKE, K. (1958). C-12-Hydroxylierung von Herzsteroiden. *Naturwissenschaften*, **45**, 366.

REPKE, K. (1959a). Glykosidspaltung im Tierkörper. *Naturwissenschaften*, **46**, 173–174.

REPKE, K. (1959b). Über Beziehung zwischen Stoffwechsel und Kumulation von Herzglykosiden. *Arch. exp. Path. Pharmak.*, **236**, 242–245.

REPKE, K. (1959c). Über Spaltung und Hydroxylierung von Digitoxin bei der Ratte. *Arch. exp. Path. Pharmak.*, **237**, 34–48.

REPKE, K. (1959d). Die Bis- und Monodigitoxoside des Digitoxigenins und des Digoxigenins: Metabolite des Digitoxins. *Arch. exp. Path. Pharmak.*, **237**, 155–170.

REPKE, K. (1963). Metabolism of cardiac glycosides. *Proc. First Int. Pharmac. Meeting*, **3**, 47, 1961. Oxford: Pergamon Press.

REPKE, K. (1966). Biochemie und Klinik der Digitalis. *Internist*, **7**, 418–426.

RUIZ-TORRES, A. (1970). Kinetik der Herzsteroide im Organismus des Menschen und der Versuchstiere. *Klin. Wschr.*, **48**, 257–270.

SCHOLTAN, W., SCHLOSSMANN, K. & ROSENKRANZ, H. (1966). Bestimmung der Eiweissbindung von Digitalispräparaten mittels der Ultrazentrifuge. *Arzneimittel-Forsch.*, **16**, 109–118.

SCHOLTAN, W. (1968). Die hydrophobe Bindung der Pharmaka an Humanalbumin und Ribuonucleinsäure. *Arzneimittel-Forsch.*, **18**, 505–517.

SEIPEL, H., HUEBER, E. F., DEUTSCH, E., LUTZ, U., WICHTL, M. & JENTZSCH, K. (1968). Quantitative Digitoxin-Bestimmung im Blut routinemässig digitalisierter Patienten. *Klin. Wschr.*, **46**, 1257–1260.

STAHL, E. (1967). *Dünnschichtchromatographie*. Berlin-Heidelberg-New York: Springer Verlag, pp. 333.

WILSON, W. S. (1969). Metabolism of digitalis. *Prog. cardiovasc Dis.*, **11**, 479–487.

WONG, K. C. & SPRATT, J. L. (1963). Assay of radioactive digoxin in liver tissue. *Biochem. Pharmac.*, **12**, 577–579.

WRIGHT, S. E. (1960). *The Metabolism of Cardiac Glycosides*. Springfield, Illinois: Charles C. Thomas, pp. 69.

(*Received August* 24, 1970)