RESEARCH PAPERS

THE METABOLISM OF OUABAIN IN THE RAT

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The urinary and biliary excretion of ouabain by rats has been estimated by paper chromatographic, colorimetric and biological methods of assay. After a dose of 1 μg./g., ouabain was excreted to the extent of approximately 90 per cent in bile and 4 per cent in urine. No metabolites of ouabain retaining the unsaturated lactone ring could be detected at this dose, but after extremely high doses traces of such metabolites were present. The influence of the polarity of the molecule on its excretion is discussed.

OUABAIN (g-strophanthin) is one of the most rapidly eliminated cardiac glycosides, but little is known about the mechanism of its destruction or excretion. Hatcher and Eggleston[1] examined its fate in the rat, which is highly resistant to ouabain, and concluded that the liver played an important role in its elimination. Farah[2], also using rats, estimated by biological methods that 80 to 85 per cent of the glycoside was excreted in the bile 2–4 hours after administration. There is, however, no information available as to whether the glycoside is excreted unchanged or as a metabolite, and no quantitative estimation of the amount excreted in urine has been published.

In this study we have re-examined the biliary excretion of ouabain in rats, using paper chromatography to separate and identify the excretory products, and estimated the amounts excreted in bile by colorimetric methods. The urinary excretion of the glycoside has also been examined by paper chromatography and the embryonic chick heart assay method of Lehman and Paff[3].

METHODS

Biliary Excretion

Male albino rats (300–450 g.) were anaesthetised with urethane and the bile duct cannulated with the shaft of a hypodermic needle attached to fine polythene tubing. Doses of 1 μg./g. were injected into the femoral vein and the bile collected for 5 hours (average yield = 3 ml.).

Urinary Excretion

Male albino rats (250–350 g.) were injected intraperitoneally with ouabain at doses of 1 or 2 μg./g. and the overnight sample of the urine of each pair of rats was precipitated with lead acetate[4] before extraction.

Extraction of Ouabain

Because of the high water solubility of ouabain it was necessary to saturate aqueous solutions with ammonium sulphate before continuous

extraction in a liquid-liquid extractor with 10 per cent methanol in chloroform for 6 hours. Using this method, recoveries from water were never less than 91 per cent.

From bile. The bile from one rat was diluted to about 15 ml. with water and extracted with chloroform in a liquid-liquid extractor for $1\frac{1}{2}$ hours to remove chloroform-soluble bile constituents which interfered with subsequent paper chromatography. Trial experiments showed that no ouabain or recognisable metabolite was removed under these conditions. The diluted bile was then fully saturated with ammonium sulphate and re-extracted with 10 per cent methanol in chloroform for 6 hours. The chloroform-methanol extract was evaporated to a small volume for paper chromatography.

From urine. After treatment with lead acetate urine was saturated with ammonium sulphate and extracted for 6 hours with 10 per cent methanol in chloroform. The chloroform-methanol extract, 30 ml., was then shaken with 3 successive quantities of 10 ml. of water to remove the oubain and leave toxic substances in the chloroform. The aqueous layers were combined and evaporated at low temperature and the residue dissolved in a small volume of methanol for paper chromatography.

Paper Chromatography

The system of Schenker, Hunger and Reichstein[5], consisting of water saturated with butanol, was used for bile and urine extracts.

Bile extracts. The chloroform-methanol extracts containing the glycoside and much pigment were streaked quantitatively across strips of Whatman paper No. 3 ($1\frac{1}{4}$ in. by 15 in.) and developed with the butanol-rich phase for 24 hours by the downward method. A longitudinal strip $\frac{1}{4}$ in. wide was cut from the chromatogram and used to locate the glycoside with alkaline *m*-dinitrobenzene[6]. The corresponding areas in the remainder of the strip were cut away, dried at 60° for 1 hour and eluted in a small soxhlet extractor with 30 ml. of methanol. The methanol was then removed, and the residue estimated colorimetrically.

Urine extracts. The extract of the urine of two rats was streaked on to strips of Whatman paper No. 4 ($1\frac{1}{4}$ in. by 15 in.) previously saturated with the aqueous phase. The paper was developed with the butanol-rich phase in horizontal tanks at 20–24° for 12 hours. The glycoside bands were located as described for bile and the areas of the chromatograms containing active material were dried at 60° for 1 hour and eluted with methanol. The methanol was removed and the residue used for biological estimations.

Estimation

In bile extracts. Because of the comparatively large amounts of glycoside extracted in bile it was possible to use colorimetric methods of estimation. The Raymond reagent was used according to the method of Anderson and Chen[7], but modified as follows. The eluate from the

chromatogram was dissolved in 5 ml. of ethanol and a convenient aliquot, usually 3 ml., of this solution was pipetted into a colorimeter tube. Then 0·5 ml. of a 1 per cent ethanolic solution of *m*-dinitrobenzene was added the tube cooled in an ice bath and 0·5 ml. of 20 per cent aqueous sodium hydroxide added. The solution was thoroughly mixed and retained in the ice bath for 10 minutes by which time the blue colour reached a maximum and remained stable for 1–2 minutes. The optical density was measured using an E.E.L. colorimeter (red filter 607). Standards were prepared and read at the same time under the same conditions.

In urine extracts. The small amount of cardioactive material present in the extracts of the urine of each pair of rats could not be estimated colorimetrically. The urine extract was diluted with Ringer's solution to give an expected ouabain concentration of about 0·5–1 μg./ml. and estimated by the embryonic chick heart method of Lehman and Paff[3].

Recoveries

Although complete recoveries using known quantities of ouabain were not obtained, results (Table I) were sufficiently consistent to allow the mean recovery figures obtained for bile (83 per cent—limit of error 78–88 per cent, P = 0·95) and for urine (58 per cent—limit of error 53–63 per cent, P = 0·95) to be used as correction factors to obtain a close approximation of the ouabain content of the bile or urine obtained in extraction experiments.

TABLE I

RECOVERY OF OUABAIN FROM BLANK BILE AND URINE

Bile			Urine		
Ouabain added (μg.)	Ouabain recovered (μg.)	Recovery per cent	Ouabain added (μg.)	Ouabain recovered (μg.)	Recovery per cent P = 0·95
400	323	81	100	60	57–63
400	346	87	100	61	57–65
400	319	80	100	51	46–56
400	292	73	100	66	60–72
400	352	88	100	59	54–64
400	346	87	100	49	43–55
Mean recovery = 83 per cent (Limits of error 78–88, P = 0·95)			Mean recovery = 58 per cent (Limits of error 53–63, P = 0·95)		

RESULTS

The paper chromatograms prepared from bile and from urine extracts after doses of 1 μg. and 2 μg./g. showed the presence of one band only (see Fig. 1a). This was identified as ouabain by eluting from the paper and rechromatographing with the genuine glycoside using three different systems: butanol saturated with water[5]; butanol: toluene, 50: 50, saturated with water[5]; and tetrahydrofurane: chloroform, 50: 50, saturated with formamide[8]. The amounts of ouabain recovered from bile in the first 5 hours after injection, and from urine in the first 24 hours for both dose levels, are shown in Table II.

Some rats were given intravenous doses of 2 μg./g. but the chromatograms of the urinary extracts of these animals did not show significant differences from those obtained after intraperitoneal doses.

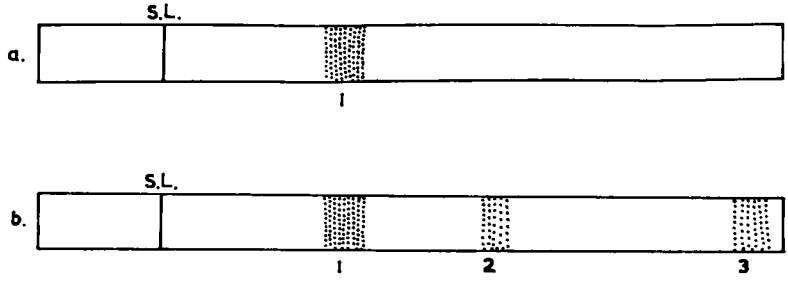

FIG. 1. (a) Chromatograms of bile extracts after doses of 1 μg./g., and of urine extracts after doses of 1 μg. and 2 μg./g. (b) Chromatograms of urine extracts after dose of 10 μg./g.

Band 1 = Ouabain.
Band 2 and 3 = Metabolites of ouabain.

When doses of 10 μg./g. were given intraperitoneally, three bands were detected with *m*-dinitrobenzene on the chromatograms of the chloroform extracts of urine (Fig. 1b). The ouabain band was very intense, but the second and third bands which appear to be metabolites of ouabain of a less polar nature were very faint. No quantitative estimations were attempted at this level, and no information was obtained about the possible nature of the metabolites.

TABLE II

EXCRETION OF OUABAIN IN BILE AND URINE

	Dose level	No experiments	Mean ouabain recovered per cent	Mean ouabain recovered (corrected) per cent	Limit of error P = 0·95
Bile	1 μg./g.	8	73	88	83–93
Urine	1 μg./g.	6	2·4	4·6	3·6–4·4
	2 μg./g.	3	2·8	4·8	4·4–5·2

DISCUSSION

These results confirm the work of Farah[2] in demonstrating that ouabain is excreted mainly in the bile and that the liver is therefore the chief organ of excretion. Furthermore, we have shown that ouabain is excreted almost entirely unchanged, as approximately 93 per cent of the dose can be accounted for in rat bile and urine as original glycoside. It is only after exceptionally high doses that metabolites of ouabain appear in urine.

The high biliary excretion of ouabain, a very polar water-soluble glycoside, is in accord with the results obtained by Cox and Wright[9] for the biliary excretion of the polar digitalis glycosides lanatoside A and lanatoside C. These glycosides have partition coefficients in favour of water and approximately 70–75 per cent of the dose is excreted in bile

without chemical modification. Ouabain, as indicated by its high water solubility and behaviour on paper chromatograms, is still more polar than the lanatosides and is excreted, also unchanged, to the extent of about 90 per cent in bile. Evidently, the polarity of the molecule is a major factor in determining the rate and extent of excretion of cardiac glycosides by the liver.

The biliary excretion of ouabain and the lanatosides (which are all non-cumulative glycosides in man) contrasts with that of the cumulative glycoside digitoxin. This glycoside is relatively non-polar and only 10 per cent of the dose can be recovered in rat bile in the 5 hours after injection[9]. Furthermore, the biliary excretory products of digitoxin in the rat consist of both unchanged glycoside and its metabolite, 12-β-hydroxy digitoxin (digoxin)[9]. There is evidence[10] that the digitoxin excreted in human bile is reabsorbed from the intestine and recirculated in the body. Some of the digoxin produced by metabolism of digitoxin may also be reabsorbed, but in the rat at least some is excreted in the faeces[11]. Ouabain, because of its highly polar nature, is not absorbed to any appreciable extent from the intestine[1] and hence would not recirculate after being excreted in bile.

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