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SHORT COMMUNICATIONS

Assay of radioactive digoxin in liver tissue *

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IN RECENT years renewed interest in the metabolism of cardiac glycosides has been noted. This reawakening of interest in glycoside metabolism has stemmed from such findings as the hydroxylation of digitoxin in the body to form digoxin[1] and the finding that the parent glycoside molecules give rise to the *mono-* and *bis*-digitoxsides in the body.[2] Studies of this type require very specific micro-methods since the therapeutic dosages of these drugs are so small. Although the present communication outlines methods for the determination of micro amounts of tritiated digoxin in liver tissue, the method should be applicable to other tissues as well. The method is based on the procedure for the determination of radioactive digitoxin,[3] chromatographically modified for the determination of digoxin. In addition, certain procedural modifications have been introduced which make the method much faster and eliminate the use of cumbersome separatory funnels.

METHODS

The tritiated digoxin used in these studies has a specific activity of 12·5 μc/mg and was generously supplied through the courtesy of Burroughs-Wellcome Ltd., Tuckahoe, N.Y. Radioactivity determinations were performed by the use of a model 703 liquid scintillation spectrometer (Nuclear Chicago Corp., Chicago, Ill.). The solvent system consisted of 5 ml 95% ethanol for the solution of the sample, and 15 ml of toluene. The total counting volume contained 4 g/l. 2,5-diphenyloxazole (PPO) and 50 mg/l. 1,4-b*is*-2(5-phenyloxazolyl)-benzene (POPOP).

The technique of liquid–liquid extraction of the glycoside from tissues is essentially that of Okita *et al.*[3] The volume relationships shown in Fig. 1 are slightly different from those of the original procedure. In addition, we find that the use of glass-stoppered centrifuge tubes in place of cumbersome separatory funnels eliminates the constant hazard of sample loss. We have routinely used 10-min periods for both shaking (adapted International Bottle Shaker) and centrifugation (1,750 rpm) of the conical-tip, glass-stoppered centrifuge tubes. The procedure was carried out with 5-ml samples of 10% liver homogenates or their equivalent in liver slices.

The chromatography procedure of Okita *et al.* for digitoxin[3] was modified for digoxin recovery. The same alumina (Alcoa F-20) and glass columns (0·5 × 14 cm) are used. The drug residue from extraction is placed on the column with two consecutive 10-ml aliquots of 2% absolute ethanol in chloroform. This is followed by 30 ml of chloroform, and both solvents are collected as the first fraction. The second fraction consists of 50 ml 5% absolute ethanol in chloroform (digoxigenin), and the last fraction consists of 25 ml of a 1:3 mixture (v/v) of 95% ethanol plus chloroform (digoxin and its *mono-* and *bis*-digitoxosides).

Each of the extraction and chromatography fractions except for the original protein residue is taken to dryness, prepared in the solvent system described above, and its radioactivity determined.

RESULTS AND DISCUSSION

Various extraction and chromatography modifications were attempted before a suitable procedure was found. Recovery experiments were performed using 1-mg quantities each of non-radioactive digitoxin, digoxin, digoxigenin, digitoxose, and the *mono-* and *bis*-digitoxosides of digoxin. These nonradioactive compounds were determined colorimetrically by either the alkaline picrate method

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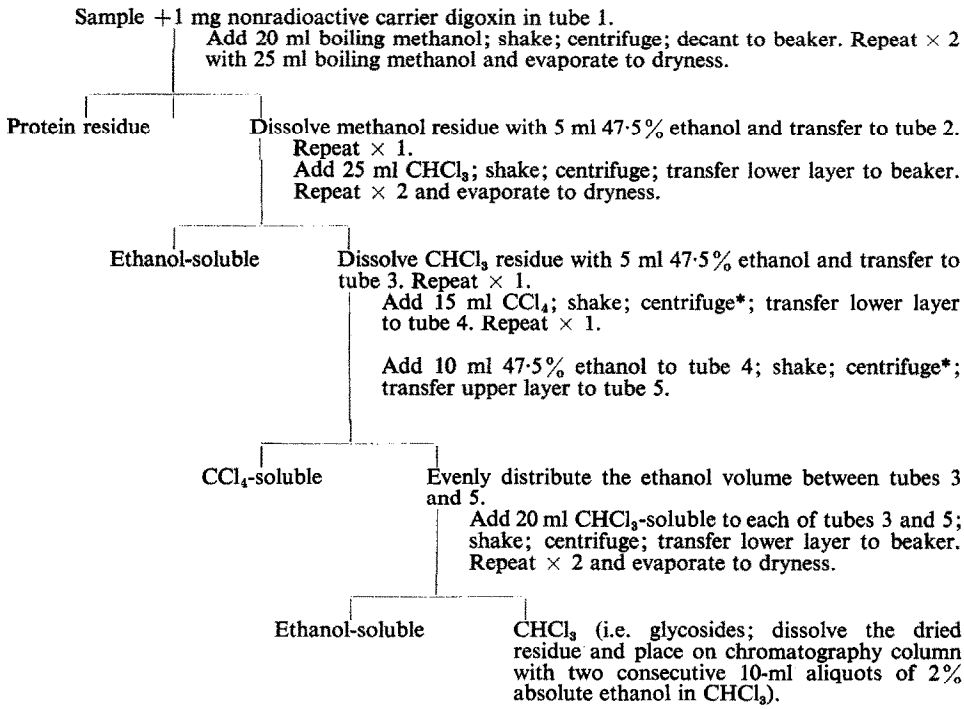

FIG. 1. Extraction of digoxin and digitoxin from liver tissue.

* If separation not complete, place unstoppered tube in 50–60° water bath.

or the Keller–Kiliani method as outlined by Rowson.[4] The colorimetric procedures were adequate to evaluate purified components, such as those from the chromatography column. However, tissue constitutents interfered with the colorimetric evaluation of various intermediate steps in the extraction procedure. The recoveries from the liquid extraction scheme as outlined are shown in Table 1. Radioactive digoxin was also quantitatively isolated by these methods.

TABLE 1. PERCENTAGE RECOVERIES OF NONRADIOACTIVE GLYCOSIDES (1 MG) FROM FRACTIONS OF THE EXTRACTION PROCEDURE (FIG. 1) AND COLUMN CHROMATOGRAPHY*

Precolumn fractions			Column fractions		
Compound	Combined ethanol-soluble	CCl_4-soluble	$CHCl_3$ soluble	Digoxigenin	Digoxin + digitoxosides
Digitoxose	98·1 ± 1·1				
Digoxigenin				97·8 ± 0·9	
*Mono*hexose digitoxosides					96·2 ± 0·8
Bis-hexose digitoxosides					97·0 ± 0·8
Digoxin					95·8 ± 0·7

* Each entry represents the mean ± standard deviation of 6 experiments. Colorimetric determinations were evaluated by methods as outlined by Rowson.[5]

The chromatography procedure was established after evaluating various sequences of different solvents and quantitatively determining the contents of 5-ml eluate fractions. Digoxigenin was recoverable in the first 25 ml of the 5% ethanol in chloroform eluate in six separate colorimetric determinations. Likewise, digoxin and its *mono-* and *bis*-digitoxosides were recovered in the first 10 ml of the 1:3 mixture (v/v) of 95% alcohol plus chloroform in six colorimetric determinations (Table 1).

In addition to the nonradioactive determinations, numerous extractions of tritiated digoxin from liver incubations have been performed in this laboratory.[5] Recoveries from 48 individual control experiments (both with and without tissue present) have yielded a mean of 95·62 ± 1·76 per cent of the original dose present in the flasks. The details of these metabolism studies will be made available in the near future.

Two precautions in regard to the chromatography procedure should be mentioned. We have restricted ourselves to the use of Alcoa F-20 alumina since certain other alumina preparations have been found to be unsuitable.* Likewise, care should be taken not to use chloroform that has been allowed to accumulate significant amounts of hydrochloric acid.

The method as outlined allows recovery of radiodigoxin and all metabolites except for volatile material and any metabolite not extractable from the original protein residue. The protein residue is readily available for radioactivity determinations by combustion techniques. The procedures as outlined are readily reproducible and relatively fast for the isolation of such chemically labile materials as the cardiac glycosides. It is stated that the further separation of digoxin from its *mono-* and *bis*-digitoxosides can be accomplished by paper chromatography using chloroform: isopropyl ether (9:1) on formamide-saturated paper.[6] As yet, we have not found this latter method completely satisfactory for quantitative separation when small amounts of drug are involved.

* Personal communication from G. T. Okita, Department of Pharmacology, University of Chicago, Ill., U.S.A.

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Effects of drugs on noradrenaline and 3-hydroxytyramine (dopamine) levels and on the noradrenaline to dopamine ratio in the rat brain

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The effects of centrally acting drugs on brain levels of 5-hydroxytryptamine,[1, 2] noradrenaline,[3, 4] dopamine[5] and γ-aminobutyric acid,[6] have recently been investigated in an attempt to explain their mode of action. Multiple injections of *dextro*-amphetamine lower brain noradrenaline levels in rats,[7] and rabbits,[8] while in cats *laevo*-ephedrine has no significant effect on the hypothalamic noradrenaline.[9] The experiments reported here were designed to determine whether the central