LETTERS TO THE EDITOR

The Identification of Digoxin Metabolite B (Digitoxin Metabolite C) with Digoxigenin Di-Digitoxoside

SIR,—The cardiac glycosides digoxin and lanatoside C are converted by the rat and by man to *metabolite B*, a derivative of digoxigenin (Brown and Wright, 1956; Ashley and others, 1958). Digitoxin (Brown and Wright, 1956) and acetyldigitoxin (Martin and Wright, 1960) also undergo metabolism in the rat to give *metabolite C* which could not be separated from *metabolite B* on several paper chromatography systems (Ashley and others, 1958) and which is therefore formed by β-hydroxylation of the steroid nucleus of digitoxin at position 12. Using paper chromatography Brown and Wright (1956) could not separate *metabolite B* from *metabolite X* obtained from rat urine after digoxigenin administration and therefore believed that *metabolite B* did not contain digitoxose. Repke, Roth and Klesczewski (1959) however obtained a metabolite (compound 8) after digitoxin administration to rats which behaved similarly to *metabolite B* (= *metabolite C*) of Brown and Wright (1956) on paper chromatograms. This metabolite gave a positive xanthydrol test for digitoxose and could not be separated from digoxigenin di-digitoxoside by paper chromatography.

Confirmation that *metabolite B* obtained from digoxin is identical with digoxigenin di-digtoxoside has now been obtained by the use of radioactively labelled ^{14}C digoxin.

Biosynthetically labelled ^{14}C-digoxin (specific activity $3{\cdot}55 \times 10^5$ c.p.m./mg.) 0·19 mg. diluted with 0·3 mg. of non-radioactive digoxin was injected into a femoral vein of each of four anaethetised rats and the bile collected through the cannulated bile ducts for 5 hr. The metabolites in the bile were separated by paper chromatography (Cox and Wright 1959) and the *metabolite B* area on the chromatogram eluted with methanol. To the eluate was added 21 mg. of digoxigenin di-digitoxoside prepared by the method of Haack, Kaiser and Spingler (1956). The recovered digoxigenin di-digitoxoside was recrystallised to constant specific activity from acetone-light petroleum (b.p. 60–80°) and specific activities of 377, 394, 404, 409 c.p.m./mg. were obtained in four separate recrystallisations. The melting point of the recovered digoxigenin di-digitoxoside (219–222°) was undepressed by a sample of this substance kindly supplied by Dr. E. Haack.

It may be concluded that lanatoside C and digoxin undergo progressive loss of sugar residues in the rat and in man to give digoxigenin di-digitoxoside. The same metabolite is produced from digitoxin and acetyl digitoxin by C(12)-hydroxylation and the loss of one molecule of digitoxose or acetyl digitoxose. The nature of *metabolite X* found in rat urine after digoxigenin administration is being re-investigated.

The author wishes to acknowledge the careful technical assistance of Mr. W. Golder in this investigation and a gift of ^{14}C-digoxin from Burroughs Wellcome & Co. (U.S.A.) Inc.

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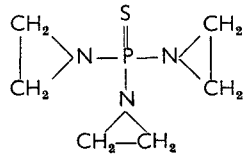
Estimation of Thiotepa in Urine

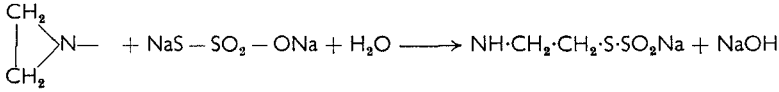
SIR,—When given in doses sufficient to suppress malignant tumours, Thiotepa has been shown to cause a severe, and sometimes fatal, depression of the bone marrow (Cree, 1960). For this reason topical and localising methods of treatment have been sought and applied with some success (Abel, 1960). In order to assess the possible systemic effects of treating bladder tumours by instilling Thiotepa directly into the bladder, it was necessary to estimate the quantity of the drug introduced, and that obtained by washing out the bladder, usually three hours later (Jones and Swinney, 1961). Because variations in technique and application may require a reassessment of this factor, and since the chemistry of the analytical reaction is unusual, the method used in these estimations is briefly reported.

Principle of Method

Thiotepa is *NN'N''*-triethylene thiophosphoramide.



Whilst information on its chemistry is largely unpublished, the ethylene-imino ring group is known to be rapidly attacked by nucleophilic reagents, of which thiosulphate is particularly suitable for reactions in aqueous solution (Golumbic, Fruton and Bergmann, 1946). The reaction which is catalysed by an acid medium (Ross, 1950, see. p. 2269), results in the liberation of sodium hydroxide equivalent to the number of ethylene imino rings attacked.



The estimation is therefore made by dissolving the drug in thiosulphate and titrating the liberated NaOH with HCl, adding an excess to bring the mixture to approximately pH 4 (methyl orange). The reaction is then allowed to go to completion (30 min.) and the amount of acid still unneutralised estimated by titration with NaOH to pH 8 (phenolphthalein).

Method

The concentrations of Thiotepa used in this study were about 1–2 mg./ml., and samples of 2–5 ml. were taken for analysis. The sample was extracted three times with 2 ml. of chloroform in a 10ml. stoppered tube. In the