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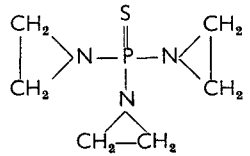
Estimation of Thiotepa in Urine

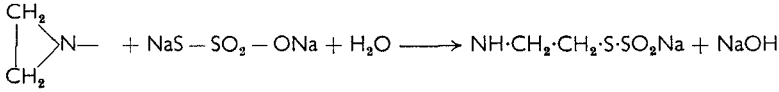
SIR,—When given in doses sufficient to suppress malignant tumours, Thiotepa has been shown to cause a severe, and sometimes fatal, depression of the bone marrow (Cree, 1960). For this reason topical and localising methods of treatment have been sought and applied with some success (Abel, 1960). In order 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Whilst information on its chemistry is largely unpublished, the ethylene-imino ring group is known to be rapidly attacked by nucleophilic reagents, of which thiosulphate is particularly suitable for reactions in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

presence of urine it was usually necessary to separate the layers by centrifugation. The combined extracts were then evaporated to dryness *in vacuo* at room temperature, and to the residue, 10 ml. of 20 per cent sodium thiosulphate solution, and one drop of methyl orange were added. The solution was then titrated with 0·1N HCl until the indicator remained red for 10–15 sec. (V_1). 30 min. later two drops of phenolphthalein were added, and the solution titrated to a pink colour with 0·1N NaOH (V_2). A blank estimation was made by treating 10 ml. of thiosulphate in a similar way (B_1 and B_2). Then $(V_1 - V_2) - (B_1 - B_2)$ = volume of 0·1N HCl equivalent to the NaOH liberated by the reaction. Since each molecule of Thiotepa (mol. wt. 189·3) contains 3 ethylene imine rings, multiplication of this volume by 18·93/3 gives the number of mg. of Thiotepa in the sample taken.

As pure crystalline Thiotepa was not available it has not been possible to check every aspect of the analytical procedure which must therefore form the basis for further study. However, estimations of the drug in standard ampoules were consistent with the stated content and several analyses of a solution kept at −15° over a week gave reproducible results.

I am greatly indebted to Lederle Laboratories through the courtesy of Dr. J. R. Wilson for unpublished information on which this method was based. I also wish to acknowledge the help of Dr. B. E. Tomlinson of the Department of Pathology, The General Hospital, Newcastle upon Tyne, in whose department these studies were made.

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The Action of Aryloxyaliphatic Acids on the Permeability of Blood Vessels

SIR,—It has been shown recently that certain aryloxyaliphatic acids have anti-inflammatory properties (Northover and Subramanian, 1961) and it was of interest, therefore, to study the correlation of chemical structure and biological activity in this series.

The method for measuring the effect of drugs on the permeability of peritoneal blood vessels has been described in detail elsewhere (Northover, 1962). Briefly, the method consists of following the movements of azovan blue dye from the circulation into the peritoneal fluid of mice given 4 ml. of 0·9 per cent saline solution intraperitoneally. The concentration of dye in the peritoneal fluid at the end of 1 hr. is a measure of the permeability of the peritoneal blood vessels to plasma albumin. Male mice weighing between 25 and 30 g. were arranged in groups of 12 animals each, and the groups were treated with graded doses of the substances under test. 0·1 ml. of a neutralised solution or suspension of the substance under investigation was administered subcutaneously to each animal. 30 min. later, 4 ml. of 0·9 per cent saline solution at 38° were given intraperitoneally to each mouse. Immediately afterwards each animal was