4. W. M. L. Baird and A. M. Reid, *Br. J. Anaesth.* **39,** 775 (1967).
5. T. M. Spoigh and G. S. Avery, *Drugs* **4,** 163 (1972).
6. A. Nana, E. Cardan and M. Cucuianu, manuscript in preparation.
7. R. L. Katz, *Anesthesiology* **35,** 602 (1971).
8. S. M. Kirpekar, I. I. Lewis and T. C. Muir, *Biochem. Pharmac.* **11,** 937 (1962).
9. J. P. Long, in *Handbook of Experimental Pharmacology* (Ed. G. B. Koelle), Vol. XV, p. 374. Springer, Berlin (1963).
10. G. L. Ellman, K. D. Courtney, V. Andres and R. M. Featherstone, *Biochem. Pharmac.* **7,** 88 (1961).
11. M. Dixon and S. E. Webb, *Enzymes*, 2nd edn, p. 327. Longmans, Green, London (1966).
12. A. D. Webb and R. L. Johnson, *Biochem. Pharmac.* **18,** 2153 (1969).

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Behaviour of an aziridine alkylating agent in acid solution

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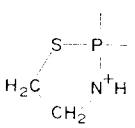
Tris(1-aziridinyl)phosphine sulphide (thiotepa) I, a biological alkylating agent used clinically in the treatment of breast cancer, is inactive when administered orally. It is unstable at pH 4·2 at 37°[1,2] and the rapid inactivation of a related compound, 2,4,6-triaziridinyltriazine, in acid solution is also recorded.[3] At low pH thiotepa is said to undergo an intramolecular alkylation giving a ring structure of the type.[4]



Since we had already observed the instability of thiotepa in acid solution it was decided to try to obtain additional evidence for the intramolecular alkylated product.

Isotopically labelled compounds, ^{35}S thiotepa, ^{32}P thiotepa and ^{36}Cl sodium chloride were obtained from the Radiochemical Centre, Amersham. Unlabelled thiotepa was supplied by Lederle Ltd. 1 ml M HCl was added to 20 ml of solutions of thiotepa in 0·2 M NaCl.[4] Changes in pH of these solutions were followed using a glass electrode and samples were removed at fixed time intervals for chromatographic examination. Thin-layer chromatography investigations were performed on silica gel plates using *n*-butanol saturated with ammonia. Spots were visualized using 5% 4-(4-nitrobenzyl)pyridine (NBP) in acetone and Ninhydrin. Radioactivity was detected using a geiger counter mounted on a travelling microscope base and autoradiography. The presence of hydrogen sulphide was confirmed using the British Pharmacopoeia arsenic limit test apparatus[5] but omitting moistening of the cotton wool with lead acetate solution and replacing the mercuric chloride paper with lead acetate paper.

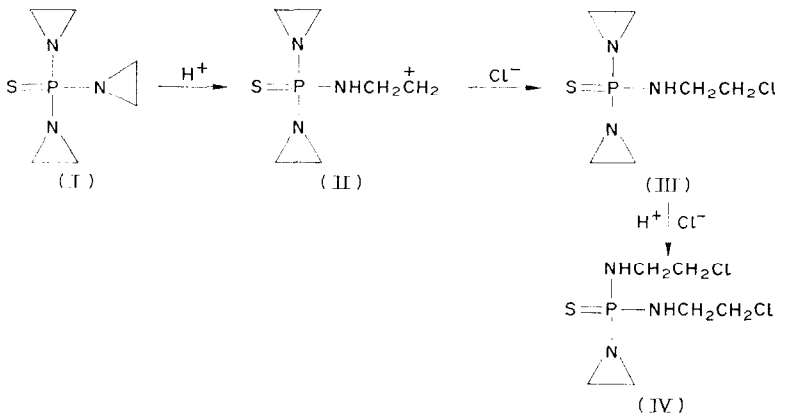
Aqueous thiotepa solutions produce a single, blue spot (R_f 0·67), visualized using NBP. Solutions of thiotepa in saline produced two spots (R_f 0·67 and R_f 0·77), both of which were NBP positive and therefore contained an alkylating function. When ^{32}P thiotepa (1 mg/ml) in saline was used, these two spots were radioactive. However, when non-radioactive thiotepa solutions were prepared in ^{36}Cl saline only the spot (R_f 0·77) was radioactive, suggesting a chloro-derivative of thiotepa. This has been shown to be the monochloro-derivative III.[6]

Acidification of thiotepa solutions in saline was followed by a concentration dependent pH rise. For example, solutions containing 5 mg/ml thiotepa showed a pH rise from 1·5 to 6·0. In contrast,

acidified aqueous thiotepa solutions showed only a slight pH shift of 0·7 pH units. The solutions became turbid and hydrogen sulphide was evolved. Using ^{35}S thiotepa most of the sulphur was transferred to the lead acetate paper as hydrogen sulphide.

The acidified solutions were examined by t.l.c. from 3- to 40-min after acidification and showed nine NBP sensitive spots [(1) R_f 0·05, (2) R_f 0·11, (3) R_f 0·18, (4) R_f 0·30, (5) R_f 0·36, (6) R_f 0·42, (7) R_f 0·67 ,(8) R_f 0·75, (9) R_f 0·81] and three NBP insensitive spots. Using authentic substances as controls, spot 4 (R_f 0·30) was identified as tepa, spot 7 (R_f 0·67) as thiotepa and spot 8 (R_f 0·75) as compound III. The intensity of the thiotepa spot diminished with time and the intensity of the tepa spot increased. Spot 9 (R_f 0·81) gradually increased in intensity. Since III was more soluble in *n*-butanol/NH_3 than thiotepa it is possible that the dichloro-derivative would be even more soluble. It is therefore suggested that spot 9 is IV. The simultaneous presence of III and IV suggests that the reaction is a two stage process.

It is probable that the acid catalysed reaction of thiotepa with chloride ions is similar to that reported for thiosulphate ions.[3] The reaction may be represented by:



The limiting factor in these experiments was the amount of acid present. Thus all the added acid was used up by 4·7 mg ml^{-1} thiotepa, corresponding to the stoichiometric requirements of two aziridine rings. Reaction of the third aziridine ring only occurred with higher acid concentrations.

It is likely that spots 5 and 6 are the analogous chloroderivatives of tepa. The combination of turbidity after acidification with an NBP positive spot close to the origin (R_f 0·05) suggests a polymerization reaction.

The chloro-derivative of thiotepa will retain alkylating ability but their reactivity will decrease. Furthermore, the modified use of NBP for the estimation of alkylating agents[7,8] is affected by chloride ions and large reductions in absorbance may be explained in terms of chloride ion competition with NBP for the alkylating agent.[6] Both these factors explain the reported[4] loss of alkylating ability in the presence of chloride ions without the need to postulate an intramolecular alkylation.

Whilst not eliminating the possibility of intramolecular alkylation, these results indicate a more likely, rapid degradation process involving the replacement of sulphur by oxygen and attack on the aziridine rings, giving rise to a variety of degradation products. This may be related to the fact that thiotepa is inactive when given orally, the acid conditions of the stomach ensuring rapid decomposition

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REFERENCES

1. L. B. MELLETT and L. A. WOODS, *Cancer Res.* **20**, 524 (1960).
2. D. S. KAUSHIK, M.Sc. thesis, Bradford University (1972).
3. W. C. J. ROSS, *J. chem. Soc.* 2257 (1950).
4. C. BENCKHUIJSEN, *Biochem. Pharmac.* **17**, 55 (1968).
5. *British Pharmacopoeia*, pp. 1242–1246. Pharmaceutical Press, London (1968).
6. C. G. BUTLER, D. S. KAUSHIK, J. MAXWELL and J. G. P. STELL, *J. Mond. Pharmac.* **4**, 359 (1967).
7. J. EPSTEIN, R. W. ROSENTHAL and R. J. ESS, *Analyt. Chem.* **27**, 1435 (1955).
8. R. TRUHAUT, E. DELACOUX, G. BRULE and C. BOHUON, *Clin. Chim. Acta.* **8**, 235 (1963).

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Levels of nucleoside and nucleotide kinases in Rhesus monkey tissues

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THE NUCLEOSIDE and nucleotide kinases are involved in the phosphorylation of many analogues of chemotherapeutic interest. Consequently, the specificity of these enzymes with analogue substrates has been the subject of considerable investigation. However, there are no reported data which deal with the relative levels of these enzymes to which analogues would be exposed *in vivo*. Such information seemed an essential counterpart to the specificity data if a reasonable assessment of the quantitative significance of various routes of analogue metabolism was to be made. It was for this reason that this study was undertaken.

Two known mammalian nucleoside kinases act on purine nucleosides. One of these, adenosine kinase (EC 2.7.1.20), has been well characterized.[1,2] The other, only recently described,[3] acts on both deoxyguanosine and deoxyadenosine. Evidence has been reported which suggests that some mammalian cells very slowly phosphorylate inosine;[4,5] however, the independence of this activity from the above-mentioned nucleoside kinases has not been established. A previous study employed 6-methylthiopurine ribonucleoside as the substrate to determine the tissue levels of adenosine kinase.[6] In the present study, the natural substrate, adenosine, was used in the presence of the adenosine deaminase inhibitor, erythro-9-(2-hydroxy-3-nonyl)adenine. This compound, a generous gift of Dr. H. J. Schaeffer of these laboratories, is a potent inhibitor of adenosine deaminase from a number of different sources.* At the concentrations used in these experiments, it was found not to inhibit a purified preparation[1] of adenosine kinase, while completely inhibiting the deaminase. Further, complete inhibition of the deaminase in the tissue extracts was demonstrated by chromatography of reaction mixtures in a solvent[7] capable of separating adenosine and inosine.

In the presence of this inhibitor, adenosine kinase was detectable in all of the monkey tissues assayed (Table 1). Liver exhibited the highest activity. With the monkey tissue extracts, the levels of adenosine kinase were very much higher than those of the other purine nucleoside kinases assayed. Phosphorylation of deoxyguanosine was detectable only with kidney extracts while phosphorylation of inosine was not detectable with any extract (Table 1).

The two pyrimidine nucleoside kinases studied were uridine kinase (EC 2.7.1.48) and thymidine kinase (EC 2.7.1.21). There is yet another pyrimidine nucleoside kinase in mammalian tissues, deoxycytidine kinase, but its relationship to the activity toward deoxyguanosine is uncertain.[12–14] Uridine kinase was detectable in six of the 13 tissue extracts assayed, while thymidine kinase was detectable only in liver and brain extracts (Table 1). Again, the levels of both these enzymes were low as compared with those for adenosine kinase. Previous studies have shown that the bulk of these kinases are present in supernatant fractions of tissue homogenates.[15]

In mammals, at least three distinct enzymes catalyze the phosphorylation of purine nucleotides. Two of these enzymes are relatively specific for AMP as the phosphoryl acceptor but differ in their

*H. J. Schaeffer and C. F. Schwenden, personal communication.