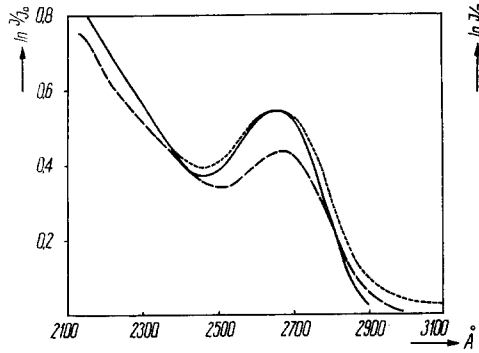

Fig. 1. Cytosine at pH 7.2, non-irradiated (——), after 85 minutes irradiation (– – – –) and after heating irradiated solution 10 min at 80° (----).

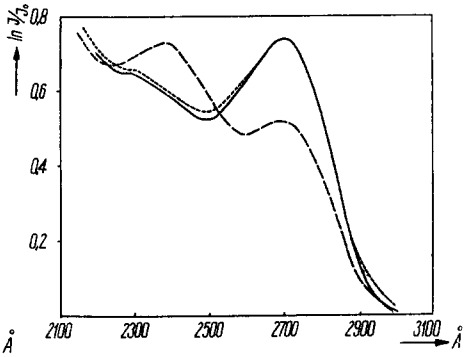

Fig. 2. Cytidine at pH 7.2, non-irradiated (——), after 20 minutes irradiation (– – – –) and after heating irradiated solution 5 min at 80° (----).

of the secondary linkages involving the pyrimidine rings. To date we have been able to obtain only indirect evidence for about 10–15% reversibility by heat with RNA in buffered medium, if the initial photolysis is performed with unfiltered radiation from a mercury resonance lamp (*i.e.* $\lambda \leqslant$ 2537 A). On the other hand a sample of apurinic acid (in which we have shown that the potentially dissociable amino and keto groups are essentially free[8]) exhibits considerable reversibility, which we attribute to the desoxycytidylic acid component. By direct hydrolysis and paper chromatography we have found that the quantum yields for photolysis of the pyrimidine nucleotide components in RNA are of the same order of magnitude as the quantum yields for the free nucleotides. In agreement with other observers we noted that the purine derivatives are, by comparison, little affected.

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Electrolytic reduction of mercaptides
A new method for the isolation of thiol amino acids and peptides

Peptides which contain a sulfhydryl group are usually isolated by precipitation as an insoluble metal mercaptide with, for example, copper or mercury. This is followed by double decomposition with H_2S, removal of the metal sulfide and recovery of the free peptide from the filtrate. This classical method is tedious and time-consuming and among its disadvantages are:

(1) Adsorption of the peptide on the metal sulfide precipitate.
(2) Dilution of the product through repeated washing of this precipitate.
(3) Decomposition during the subsequent concentration.
(4) It is frequently difficult to remove salts completely by washing of the metal mercaptide.
(5) All steps after the H_2S decomposition must be carried out in the absence of oxygen.
(6) Formation of colloidal sulfur from H_2S is often troublesome.

We have now found that mercury mercaptides of thiolamino acids and peptides can be

reduced quantitatively from the solid state by electrolysis at a mercury cathode to yield concentrated, salt-free solutions of the corresponding free thiol compounds. For a simple dimercaptide, the electrode reaction is:

$$RSHgSR + 2H^+ + 2e = 2RSH + Hg \text{ metal}$$

The apparatus used was the "Electrolytic Desalter" described by CONSDEN *et al.*[1]. The main advantages of this procedure are:

(1) In contrast to chemical methods, no excess reducing agent is present at the end of the reduction and the only by-product is metallic mercury.

(2) The thiol group is protected from oxidation since hydrogen is evolved from the cathode during the electrolysis.

(3) Neutral salts are automatically removed in the apparatus used so that only the isoelectric form of the peptide is present in solution after electrolysis.

(4) Since successive portions of the solid mercury mercaptide can be fed into the electrolysis vessel as reduction proceeds, very concentrated solutions can be obtained from which the peptide can then be crystallized directly.

The example of glutathione might be used to illustrate the procedure in somewhat greater detail:

300 mg glutathione in 100 ml water were precipitated wth 5 ml acid mercuric sulfate[2]. The resulting precipitate was separated and washed 3 times by centrifugation. A portion of the precipitate was added to 4 ml water in the electrolysis chamber of the desalter. Ice-cold H_2SO_4 (1 %) was circulated through the anode chamber. The current was kept at 0.5 amp. Dissolution of the precipitate occurs within 10–20 min, but at this stage the clear solution is still nitroprusside-negative. This is due to the fact that all the "loosely-bound" mercury, *i.e.* bound on groups other than sulfur, has been reduced and that the simple dimercaptide, $Hg(SG)_2$, is soluble[3]. After a few more minutes quantitative reduction to glutathione occurs. Further portions of precipitate were now added and dissolution occurred more quickly as the proportion of glutathione to mercury increased. The reduction of all of the mercaptide precipitate was complete in about 1 h. After this the current was allowed to drop to a constant minimum value (about 0.1 amp) which indicates that the solution is essentially salt-free. Analysis of the solution for –SH[4] showed quantitative recovery, and chromatography of the N-ethyl maleimide derivative in *n*-butanol/formic acid/H_2O[5] produced only a single spot with an R_F identical with that of the original sample of glutathione.

In view of our previous use of S-phenylmercury derivatives for the isolation and characterization of thiols[6,7], the reduction of these derivatives in the desalter was also investigated. In agreement with previous polarographic results[8], it was found that the phenylmercuric moiety is reduced smoothly to benzene, metallic mercury and some insoluble diphenyl-mercury, leaving the thiol in the aqueous phase.

Since glutathione is usually isolated as the cuprous mercaptide, this compound was also subjected to electrolysis, but its reduction was found to be slow and the concomitant formation of metallic copper contaminates the mercury cathode. The cuprous mercaptide of glutathione can, however, be easily transformed into the mercury mercaptide by treatment with acid mercuric sulfate. The mercury mercaptide can then be washed free of copper and decomposed by electrolysis as described above.

Further details of this method as well as its application to the preparation of homocysteinyl peptides will form the subject of a subsequent communication. The isolation of –SH containing proteins via the electrolytic reduction of their mercury mercaptides is also under investigation.

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