Table 1. PAPER CHROMATOGRAPHY OF HYDROPEROXIDIC DERIVATIVES OF THYMINE

Paper, Whatman No. 1; solvent, *n*-propanol/1 *N* hydrochloric acid (85/15, v/v); temperature during run, 2° C.

| Compound | | $R_F$ |
|---|---|---|
| 4-Hydroxy-5-hydroperoxythymine | *cis** | 0·51 |
| 4-Hydroxy-5-hydroperoxythymine | *trans** | 0·33 |
| 4-Hydroperoxy-5-hydroxythymine | *cis** | 0·62 |
| 4-Hydroperoxy-5-hydroxythymine | *trans** | 0·43 |
| 4-Hydroperoxy-5-bromothymine | *trans** | 0·83 |
| 4-Hydrogen peroxide* | | 0·68 |
| Thymine† | | 0·55 |

* Detected by spraying a 4 per cent alcoholic solution of potassium iodide.

† Detected in ultra-violet light.

compounds. On heating a solution of the mixture, the product with $R_F$ 0·51 is transformed into a product giving a spot with $R_F$ 0·33, while the product with $R_F$ 0·62 remains unchanged. Accordingly, it may be suggested that the product with $R_F = 0·51$ corresponds to formula II *cis*, as indicated in Table 1. These syntheses and the relationships between the compounds are summarized in the scheme on p. 58.

All the compounds can be reduced at the dropping mercury electrode in 0·1 *M* potassium sulphate at the same potential near 0 volt, against the saturated calomel electrode at 25° C. They behave similarly on 'Dowex 50–$H^+$' columns.

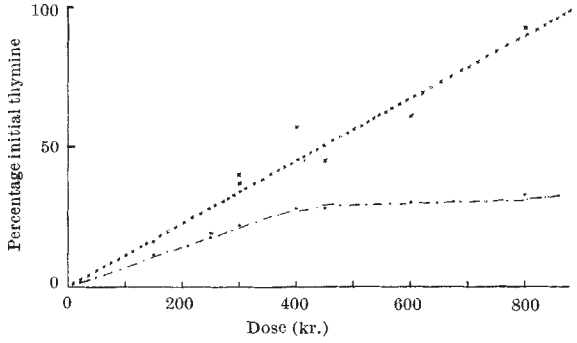


Fig. 1. Destruction of thymine (—×—×—) and total production of hydroperoxide (—·—·—) by X-irradiation of a $10^{-3}$ *M* thymine solution by X-rays of 40 kV., filtered through 0·04 mm. aluminium; dose, 25 kr., in air

In Fig. 1 are shown the curves relating dose to thymine destruction and total hydroperoxide production, during X-irradiation in air. A $10^{-3}$-*M* solution of thymine, after irradiation with 400 kr., contained $2·1 \times 10^{-5}$ *M* hydrogen peroxide and $1·9 \times 10^{-4}$ *M* hydroperoxide. After repeated lyophilizations, the residue was put on a 'Dowex 50-*X* 8' column, 1 cm. × 50 cm., in 0·1 *N* hydrochloric acid. The eluate was collected in 4-ml. fractions. Hydroperoxidic products, detected by the iodide reagent, appeared in fractions 5–11, and unchanged thymine in fractions 16–21. After paper chromatography and spraying with iodide reagent, material contained in fractions 5–11 gave a strong spot at the level of the spot given by compound I *cis* and a faint one at the level of the spot given by compound I *trans*. Control chromatographs of various mixtures of synthetic peroxides and peroxides produced by X-irradiation demonstrated that the latter cannot be distinguished from the former with corresponding $R_F$'s. Therefore, it may be suggested that X-irradiation of thymine in aqueous aerated solutions actually produces hydroperoxides I *trans* and I *cis*.

We wish to thank Dr. R. Laterjet for his interest throughout this work.

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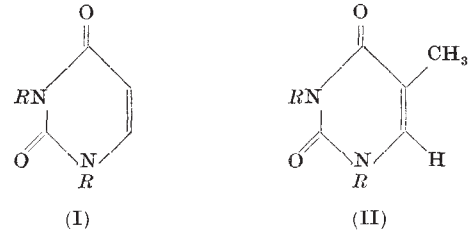
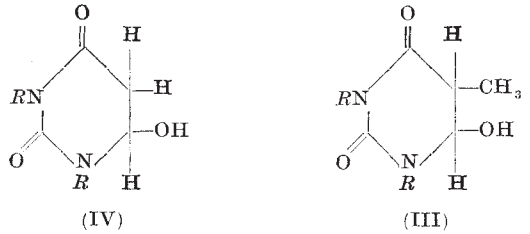
## Ultra-violet Irradiation of 1,3-Dimethylthymine

WHEN 5,6-unsubstituted pyrimidines (I) such as uracil, uridine and 1,3-dimethyluracil are irradiated with ultra-violet light, the absorption spectra gradually decrease with a simultaneous increase in end absorption. These spectra can be reversed to the original by acid, alkali or heat[1].



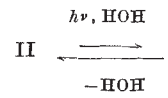

However, compounds substituted in the 5-position (II), such as thymine, thymidine and 1,3-dimethylthymine, do not show reversal under similar conditions. Most investigators have suggested that this difference may be due to totally different photochemical reaction mechanisms in the two cases[2].

Upon close examination of these two groups of compounds it appears that they probably have the same electronic distribution, because the ketonic form as shown above is probably the common and predominant configuration in both[3]. Since the interaction with ultra-violet light is related to the electronic state of a compound, it is not unreasonable to assume that the initial step is similar for both groups of compounds.

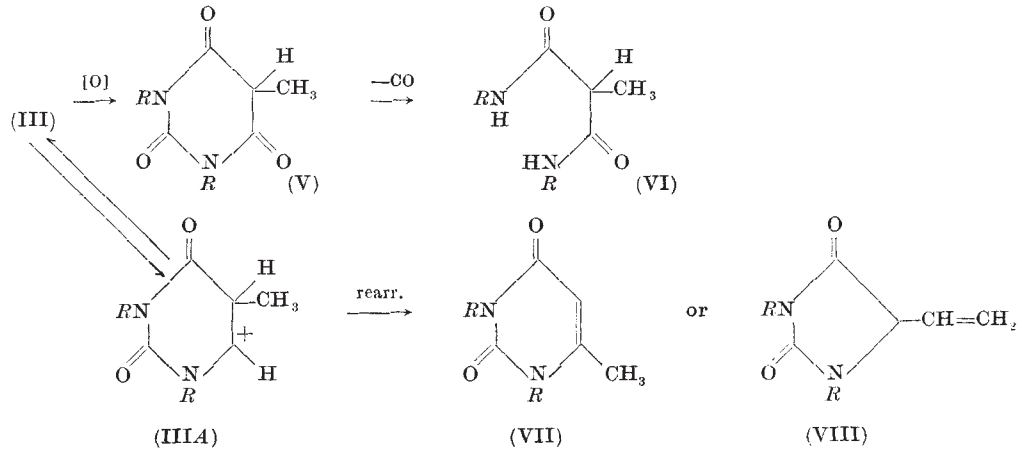



If the above assumption is true, then 6-hydroxyhydrothymines (III) would be expected as the first products, because 6-hydroxyhydrouracils (IV) have been shown to be the first products of the irradiation of uracils (I)[1]. The reconstitution reactions of uracils were found to be dehydrations[1]. For thymines, such dehydration would involve the much more reactive III°-H rather than the II°-H as in the uracils. There-

fore, the dehydration of III is probably much faster than the photo-addition of water to II. This reverse



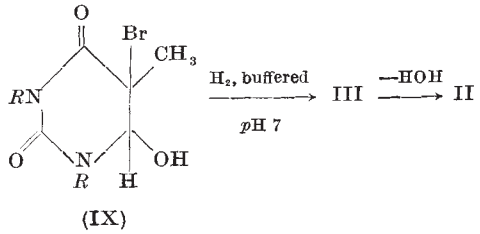

reaction would prevent the detection of III during and after irradiation, and would not be associated with a decrease in the absorption spectrum of II. Actually, however, this spectrum decreased with irradiation, and probably was due to further reactions of III to form irreversible compounds. For such reactions there are two possible routes :




If carbonium ions (IIIA) were formed from III, then through rearrangement either VII or VIII or both could be the products. If 'oxidation' were to occur, according to the route already established for uracils, then V would be the intermediate. Upon decarboxylation N,N′-dimethylmethylmalonamide (VI) would be the product[4].

In order to support experimentally the above arguments, the following two points would have to be demonstrated : first, the intermediate of hydration (III) must be shown to be much more unstable than that of 6-hydroxyhydrouracils (IV) ; second, one of the irradiation products via the intermediate (III) would have to be isolated.

We have used 1,3-dimethylthymine as a model compound. First, 5-bromo-6-hydroxy-1,3-dimethylhydrothymine (IX) was prepared and was reduced in a manner identical with that used for the preparation of 6-hydroxy-1,3-dimethylhydrouracil[1,5]. Examination of the ultra-violet spectrum of the reaction solution suggested that only 1,3-dimethylthymine was obtained as the product with little indication of the existence of 6-hydroxy derivatives.




Upon hydrogenolysis of the 5-bromo-6-hydroxy derivatives of uracils, however, the following yields of 6-hydroxy derivatives were obtained in solution : from uridine, 40 per cent ; from 1,3-dimethyluracil, 80 per cent ; and from uracil, 30 per cent. Therefore, this suggested that III has a much greater tendency for dehydration than uracils have. Second, 1,3-dimethylthymine was irradiated in aqueous solution until a flat ultra-violet spectrum was obtained. The irradiation products were then separated and purified. One of the products has been identified as N,N′-dimethylmethylmalonamide (VI, m.p. 157–158° C. Found : C, 50·08 ; H, 8·39 ; N, 19·42. Synthetic VI, m.p. 157–158° C., mixed m.p. with irradiation product 157–158° C. ; Found : C, 50·02 ; H, 8·25 ; N, 19·65. The infra-red spectra of synthetic VI and the irradiation product were identical.) On the basis of this evidence we would like to suggest that 1,4-addition of water to the thymine derivatives is the first step in the ultra-violet irradiation effect.

By examination of the quantum yields of the irradiation of thymine both in light and heavy water, Shugar has drawn the conclusion that the uptake of a water molecule is not involved[6]. From our findings it would appear that the measurements he made were actually of the subsequent slower steps and probably not for the initial fast reversible step.

Therefore, from the above findings, we have demonstrated that uracil and cytosine derivatives react similarly toward ultra-violet irradiation. This emphasized the fact that for the photochemical pathway of ultra-violet irradiation effects, the differences in electronic distributions of compounds are of more importance than the differences in their structures. They further suggest that the hydration product (III) may be of importance in photoreactivation reactions. Although the first irradiation products of uracils exhibit the phenomenon of reversibility, the irradiated uracils are stable under the customary photoreactivation conditions. Under biological conditions, however, the unstable initial thymine products (III) might be stabilized by secondary linkages, for example, H-bonds, in the nucleic acids. The H-bonds so formed could be broken by the usual photoreactivation conditions. Thus, thymines might be reconstituted and again show the biological activity of the original bases.

This work was carried out under the terms of Contract AT(30–1)911 of the Atomic Energy Com-

mission with the Physiology Department, Tufts University School of Medicine. I wish to thank M. Apicella and B. R. Stone for their able assistance.

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## Degradation of Thiotaurine by Ionizing Radiations

THE degradation of sulphur-containing compounds by ionizing radiation has been extensively studied[1–3] in view of the protective action of those compounds against radiation damage in animals. Recently, thiotaurine (aminoethylthiosulphonate) has become available for chemical and biological investigation[4,5]. Since thiotaurine was discovered as a metabolic product of cystine[6] and cystamine[7] in the rat, and since it is chemically related to cysteamine, it seemed of interest to study its reactivity towards irradiation with X-rays and γ-rays.

30 μmoles of pure thiotaurine dissolved in 3 ml. of water were placed in a glass vessel 2·5 cm. diameter. The solution was irradiated for a suitable length of time with a Philips 50 kV. X-ray source having a beryllium window. The shorter distance from the window to the centre of the solution was 1 cm. The intensity of irradiation was determined with a ferrous sulphate dosimeter[8]. 0·15 ml. of the solution was withdrawn for analysis at intervals.

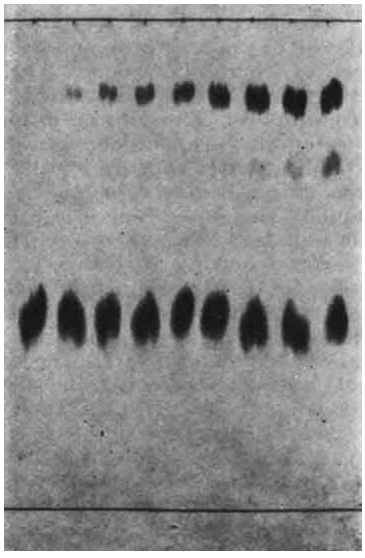


Fig. 1. Progressive chromatogram of the irradiated solution of thiotaurine with X-rays. Dose (r.), left to right, 0, 12,000, 36,000, 60,000, 84,000, 120,000, 240,000, 360,000, 480,000. Descending chromatogram in collidine–lutidine, developed with ninhydrin. 0·5 μmole of initial thiotaurine spotted at the starting line. *A*, hypotaurine; *B*, taurine; *C*, thiotaurine

As soon as irradiation started it became apparent that some reaction was taking place: the solution became more and more turbid. The degree of turbidity increased with the time of irradiation. The unirradiated control remained clear for a long time.

The material which caused turbidity was identified as colloidal sulphur by sedimentation in a 'Spinco' model *L* preparative ultracentrifuge at 125,000*g*, followed by conversion of the washed residue to thiocyanate by the procedure of Bartlett and Skoog[9].

Some of the compounds produced by the radiochemical degradation of thiotaurine have been detected by paper chromatography. At intervals a sample of the irradiated solution was spotted on a Whatman No. 4 filter paper and the chromatogram was run in collidine/lutidine/water (1:1:1 v/v) and developed with ninhydrin. Apart from a residue of unchanged thiotaurine, two main compounds reacting with ninhydrin appeared on the chromatogram. These have been identified, by careful comparison with the synthetic products and by specific reactions[6], as hypotaurine and taurine. Hypotaurine is the first degradation product to appear; its spot appears after a dose of 12,000 r. Taurine appears later and only in small amounts.

The production of hypotaurine and colloidal sulphur is consistent with the following overall reaction:

$$NH_2—CH_2—CH_2—SO_2SH \rightarrow NH_2—CH_2—CH_2—SO_2H + S$$

which represents the reversal of the reaction used for the synthesis of thiotaurine from hypotaurine and sulphur[4].

Essentially the same results have been obtained by irradiating a solution of thiotaurine with a comparable dose of γ-rays from a radium source immersed in the solution. The irradiation of a solution of thiotaurine buffered with phosphate *p*H 7·4 also gave identical results.

It is of interest that cystamine, one of the best known protective agents against radiation damage, under the same conditions and using the same procedure to detect degradation products, gave only a faint trace of taurine even with the higher doses of X-rays. In the light of these results the comparative effect of cystamine and thiotaurine in the radioprotection of animals is being studied.

This work has been assisted by a grant of the Comitato Nazionale Ricerche Nucleari.

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