The Effect of Nitrogen Mustards upon the Ultraviolet Absorption Spectrum of Thymonucleate, Uracil and Purines*

Alfred Chanutin, Ph.D., and Erland C. Gjessing, Ph.D.

(From The Biochemical Laboratory, University of Virginia, Charlottesville, Virginia)

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In a previous paper (2) it was demonstrated that nitrogen mustards caused a considerable decrease in the viscosity of sodium thymonucleate solutions. The present work was undertaken to determine whether this effect was due to an interaction of the nitrogen mustards with thymonucleate or some of its constituents.

An interaction between nitrogen mustards and thymonucleate was demonstrated but could not be correlated with the effect on viscosity.

EXPERIMENTAL

Sodium thymonucleate.—A 2 per cent solution of sodium thymonucleate (2) was diluted with an equal volume of an acetate buffer (pH 6.0; ionic strength 0.2). Nitrogen mustards were added to 50 ml. of this nucleic acid-acetate mixture and stirred for an hour at room temperature. A 2 ml. aliquot was diluted to 200 ml. with water and the ultraviolet absorption spectrum was determined in a Beckman spectrophotometer. The control solution was treated similarly but no agent was added.

In preliminary experiments it was found that tris (β chloroethyl) amine caused a shift in the absorption spectrum. Equimolar concentrations of ethyl bis (β chloroethyl) amine and methyl bis (β chloroethyl) amine did not cause a similar shift. It is assumed that the negative effect of the 2 latter compounds was due to the slow formation of active transformation products. Consequently, tris (β chloroethyl) amine was used for all experiments.

To 50 ml. of the nucleate-acetate buffer mixture were added varying amounts of tris (β chloroethyl) amine hydrochloride and the procedure described above was followed. The absorption spectra are shown in Fig. 1. The maximum absorptions for the control and the 15, 30, 63, and 125 mgm. groups are 259, 259, 260, 262, and 263 mμ, respectively. The shift in the spectrum towards the longer wave lengths becomes more pronounced with increased concentrations of the agent. No change is observed after the addition of 15 mgm. of the nitrogen mustard. Pronounced changes in viscosity of nucleic acid are seen at much lower

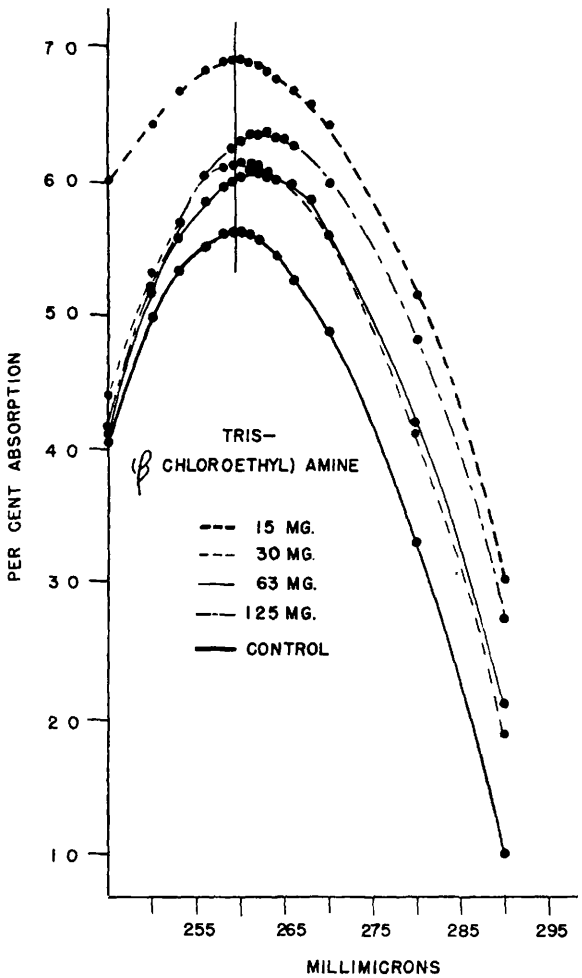

FIG. 1.—Effect of varying amounts of a nitrogen mustard on absorption spectrum of thymonucleate.

concentrations (2). Since large amounts of the agent are necessary to produce a shift in the spectrum, it is assumed that this phenomenon is not associated with the depolymerizing effect of nitrogen mustards. It is

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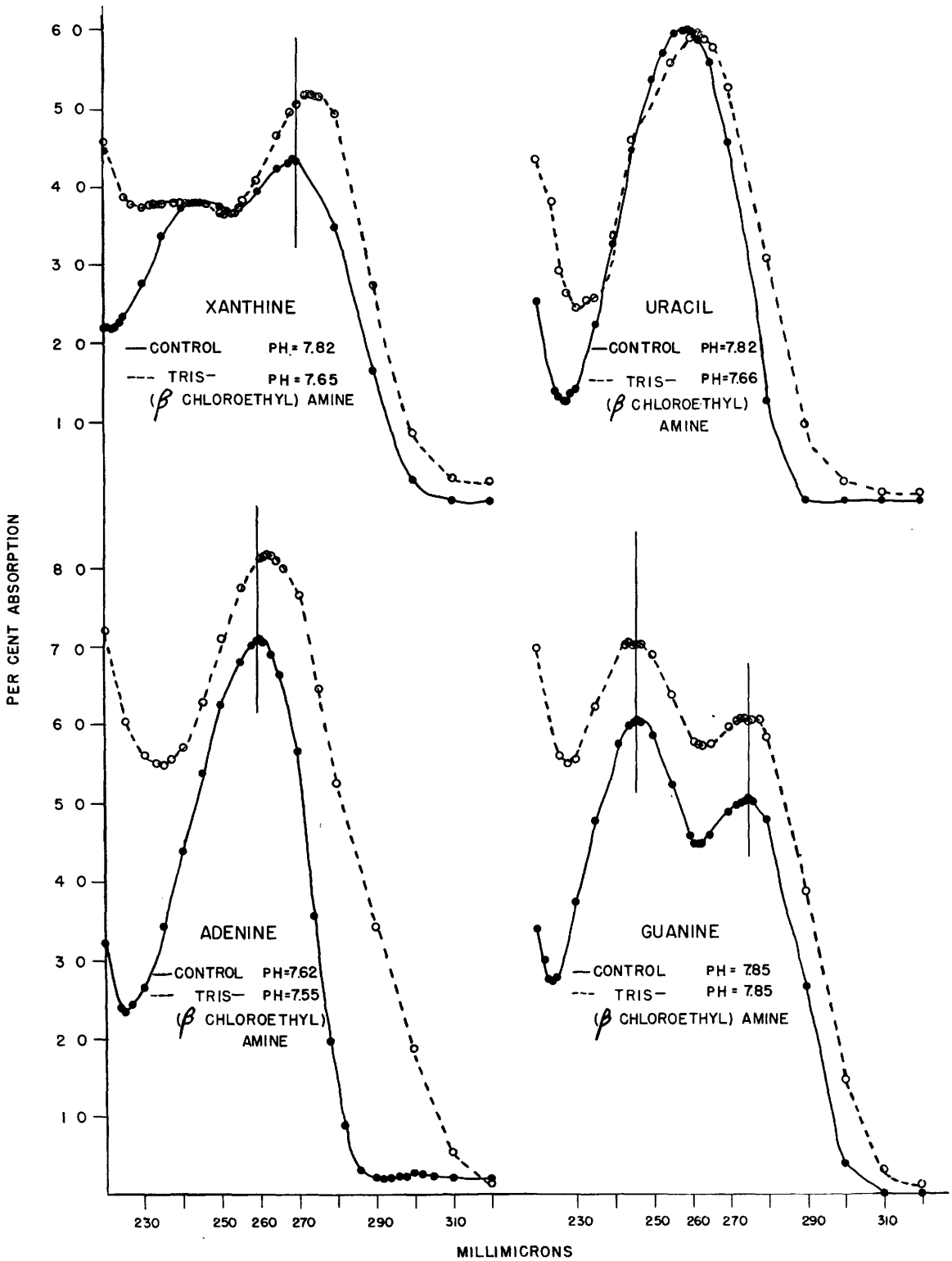

FIG. 2.—Effect of a nitrogen mustard on absorption spectrum.

well known that weighting a molecule with an aliphatic hydrocarbon chain causes a similar shift in absorption.

Purines and pyrimidine.—Solutions of adenine sulfate, $2H_2O$ (0.1 per cent); guanine hydrochloride, $2H_2O$ (0.1 per cent); xanthine (0.06 per cent); and uracil (0.06 per cent) were prepared in a borate buffer of pH 9.2. To 50 ml. of each of the above solutions were added 50 mgm. of tris (β chloroethyl) amine hydrochloride and the mixture was stirred for 1½ hours. During the stirring, the pH of the solution was maintained between pH 8 and 10 by adding ammonium hydroxide. This alkaline reaction was necessary to keep the materials in solution. To a 4 ml. aliquot, 15 ml. of phosphate buffer (pH 7.4) were added and brought to 100 ml. with water. The absorption spectra of these dilute solutions were determined.

TABLE I: EFFECT OF TRIS (β CHLOROETHYL) AMINE ON THE ULTRAVIOLET ABSORPTION SPECTRUM OF 3 PURINES AND A PYRIMIDINE

	Control	Tris (β chloroethyl) amine
	Maximum absorption, mμ	Maximum absorption, mμ
Adenine	260	262
Guanine	246, 272.5	244, 246, 274, 278
Xanthine	245, 269	273
Uracil	259	262

The absorption spectra of the control and treated samples are shown in Fig. 2. The detailed data are given in Table I. It is clear that the guanine-nitrogen mustard mixture yields a new spectrum, characterized by the appearance of two new peaks. The changes occurring after treating xanthine are pronounced, since one peak disappears and the other is shifted. There is a definite shift of the spectrum to longer wave lengths in the case of adenine and uracil.

DISCUSSION

The nitrogen mustards are capable of reacting with amino acids, peptides, proteins, pyridine compounds, adenosine, thiamin and other compounds such as inorganic and organic phosphates (1). Evidence is presented in these experiments that purines and pyrimidines also react with these agents.

At present no procedure is available to determine the group or groups in nucleic acid which react with nitrogen mustard to cause changes in the ultraviolet absorption spectrum.

SUMMARY

Spectrophotometric evidence is presented to show that the nitrogen mustard, tris (β chloroethyl) amine, reacts with sodium thymonucleate, adenine, guanine, xanthine, and uracil.

REFERENCES

1. FRUTON, J. S., and BERGMAN, M. Personal communication.
2. GJESSING, E. C., and CHANUTIN, A. The Effect of Nitrogen Mustards on the Viscosity of Thymonucleates. Cancer Research, **6**:593-598. 1946.