

Minnesota Section

University of Minnesota, May 23, 1935.

8150 C

Effect of X-Rays on Methylene Blue and on Trimethyl Thionine.

WILHELM STENSTRÖM AND HAROLD R. STREET.

From the Division of Biophysics, Department of Physiology, University of Minnesota.

When a dilute aqueous solution of methylene blue is exposed to X-rays or ultra-violet radiation, a color change is effected. This change has been utilized to determine the relative intensity of the radiation.^{1, 2} It is, however, difficult to obtain consistent results with solutions made up at different times. One reason, therefore, is undoubtedly to be found in the fact that methylene blue contains small amounts of related substances which also have strong colors. The most conspicuous of these substances is trimethyl thionine, which is present as an impurity in varying amounts up to 10% in methylene blue.³ This study was undertaken mainly to find out how the presence of trimethyl thionine may affect the color change of methylene blue.

Trimethyl thionine was synthesized according to Fierz-David's⁴ method for preparing methylene blue with the substitution of monomethyl aniline for the dimethyl aniline called for in Fierz-David's directions. There is no possibility of the presence of methylene blue in this product. The methylene blue was recrystallized several times. However, it is known that the purest methylene blue available contains a certain amount of trimethyl thionine.

The absorption curves of unirradiated methylene blue and trimethyl thionine were determined by means of a spectro-photometer.

¹ Stenström, Wilhelm, and Lohmann, Anne, *Radiology*, 1933, **21**, 29; *ibid.*, 1934, **22**, 304.

² Webster, T. A., Hill, L., and Eidinow, A., *Lancet*, 1924, **1**, 745.

³ Holmes, W. C., *Stain Technology*, 1928, **3**, 45.

⁴ Fierz-David, *The Fundamental Processes of Dye Chemistry*, J. & A. Churchill, London, 1921, p. 174.

These curves shown in the figure are very similar, but have distinct differences. The absorption of methylene blue has a peak between 6650 Å. U. and 6700 Å. U. and extends further into the red than that of trimethyl thionine which has its peak between 6500 Å. U. and 6550 Å. U.

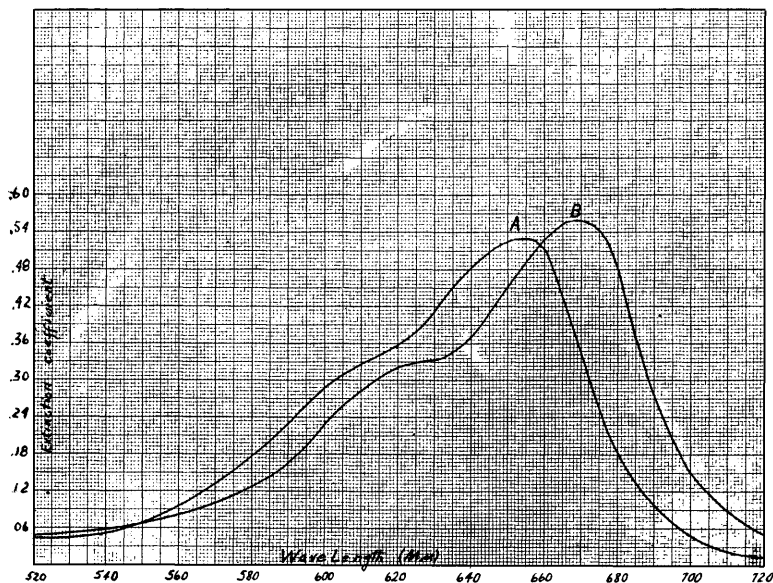


FIG. 1.

Absorption curves of methylene blue and trimethyl thionine, concentration 3 mg. per liter. (A) trimethyl thionine, (B) methylene blue.

Since in former work the change in methylene blue concentration was measured by the change in extinction coefficient at 6700 Å. U., solutions of methylene blue and trimethyl thionine were prepared which had the same extinction coefficient at 6700 Å. U. A solution of methylene blue with a concentration of 3.26 mg. per liter was used. In order to have the same absorption it was necessary to use a concentration of trimethyl thionine of 5.25 mg. per liter. When these solutions were exposed in small glass tubes to the same amount of X-rays (2460 roentgens) the absorption at different wavelengths was changed to a relatively different extent for the 2 solutions. Thus methylene blue had its maximum reduction of 21.7% at 6700 Å. U. where trimethyl thionine had a much smaller change, 12.4%. The maximum change of absorption for trimethyl thionine was at 6500 Å. U. with 14.6% reduction, at which wavelength methylene blue showed 17.5% reduction. Since in using methylene blue as indicator for X-ray intensity we have previously determined

the reduction of absorption at 6700 Å. U. it seems that the change at this wavelength will be smaller the greater the amount of trimethyl thionine present as an impurity. With a mixture of 10% trimethyl thionine and 90% methylene blue the change at 6700 Å. U. ought to be about 21% instead of 22%. In one experiment of this type no definite difference was discovered; however, this was not surprising as the difference of 1% is of the order of magnitude of the experimental error. It seems in any case that the presence of trimethyl thionine should reduce the sensitivity of the methylene blue solution to a certain extent though not enough to account for all the difference we have noticed with solutions made up at different times or from different samples of methylene blue.

8151 C

Requirement of the Flour Beetle (*Tribolium confusum* Duval) for Vitamins in the B Group.

HAROLD R. STREET AND LEROY S. PALMER.

From the Division of Biochemistry, University of Minnesota, St. Paul.

Insects offer certain possibilities for vitamin assay. If the vitamin requirements of certain insects were known it should be possible to assay vitamins with much less time and labor than with rats or similar animals. A suitable insect for nutritional research is the flour beetle, *Tribolium confusum* Duval.

When raised on whole wheat flour at 32° and 70% humidity, the larvae transform to the pupal stage in an average time of 16 days. Sweetman and Palmer¹ found that on a diet of purified food materials devoid of all vitamins the insects did not pupate. The addition of a small amount of material containing the vitamin B complex enabled the larvae to pupate in a normal time.

The renewed interest in methods for assay of the vitamins in B group brought about by the recent advances in the chemistry of these substances prompts us to report briefly experiments conducted by us in 1931-32. In this study an attempt was made to determine which members of the vitamin B complex are necessary for *Tribolium*. A basal diet of casein 15, crisco 3, Osborne-Mendel salt mixture 4, dextrin to 100 was used. Various vitamin supplements

¹ Sweetman, M. D., and Palmer, L. S., *J. Biol. Chem.*, 1928, **77**, 33.