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## The Fluorescence Spectrum of a Pigment Elaborated by the Diphtheria Bacillus.

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A pigment which is elaborated by the diphtheria bacillus has been reported by several investigators.[1-12] The fluorescence spectrum of the diphtheria bacillus (Park-Williams No. 8) cultivated on a coagulated serum has been observed by Dhéré and Rapetti. The band lies between λ623 and 609mμ, with maximum at λ616mμ. They attribute the fluorescence to the presence of coproporphyrin.

This note records the fluorescence spectrum of the acid phase of a hydrochloric-acid-treated ether extract (made after Fischer's method) from 500 cc. of a 7-day culture filtrate of *C. diphtheriæ*, strain No. 3203,[5] grown in a modified infusion-free peptone medium containing sodium acetate and maltose. The flocculating value approximated 20 Lf. One hundred cubic centimeters of the ether extract were concentrated by evaporation to 30 cc. and then treated with 5.4% hydrochloric-acid solution in the proportion of 2 parts ether extract to one part of acid solution.

The original ether extract and, after treatment with the hydrochloric-acid solution, the ether and acid phases were examined for fluorescence when irradiated with ultraviolet light from a quartz mercury-vapor arc through a red-purple corex filter (Corning) and a 4-cm. depth of 4% copper-sulfate solution.

The ether extract from the filtrate appeared a brilliant red in

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20 cm. depths and fluoresced with a brilliant red light. The ether phase of the treated ether extract appeared orange-red and fluoresced with a very faint grey-rose light which did not measurably affect a Wratten M plate exposed for one hour. The acid phase appeared a brilliant blue-violet and fluoresced with a brilliant red light.

The fluorescence spectrogram of the acid phase made on a Wratten M dry plate and the helium comparison spectrum are shown in Fig. 1, together with the microphotometric tracing of the fluorescence spectrogram.

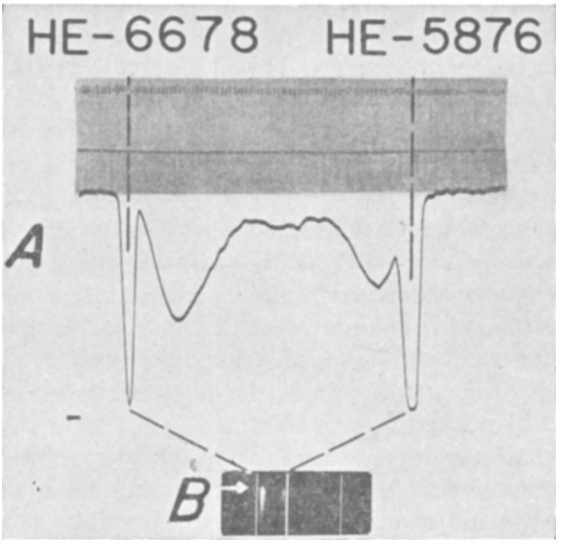


FIG. 1.

Fluorescence spectrum of the acid phase of a hydrochloric-acid-treated ether extract of a culture filtrate of *C. diphtheriæ* and the microphotometric tracing of the spectrogram.

A. Microphotometric tracing.

B. Fluorescence spectrogram with helium comparison spectrum.

Measurements of the microphotometric tracing* of the fluorescence spectrogram indicate maxima for the fluorescence bands at about λ5951, 6160, and 6502 Å. There is some evidence of "fine structure" in the bands at about λ6090 and 6280 Å. The band with the axis at λ6160 Å agrees with that observed by Dhéré and his colleagues[6, 7] in the fluorescence spectrum of diphtheria organisms. Bois's values (Atlas des spectrogrammes reproduits, p. 9)[9] for the maxima of fluorescence bands of coproporphyrin in 2 N. HCl are λ595, 620, and 653 mμ.

* The tracing was made with a Zeiss microphotometer.