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The Cytochrome of Diphtheria Bacilli and its Relation to Diphtheria Toxin.

C. B. COULTER AND F. M. STONE.

*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.**

In the study of substances that may be involved in the oxidation-reduction processes of the living cell attention is drawn to the intracellular pigment cytochrome which was originally observed by McMunn¹ and has recently been rediscovered and renamed by Keilin.² This pigment is known only by the characteristic absorption spectrum which is given by the substance in the reduced state; the oxidized form shows no selective absorption or at most a faint shading. Among bacteria this pigment is found in all aerobic forms, and is most distinct in the macroaerophiles. It is absent from the obligate anaerobes. Its occurrence in *B. diphtheriae* has been observed by Yaoi³ and Tamiya.

We have studied the occurrence of cytochrome in cultures and filtrates of different members of the diphtheria bacillus group. In masses of bacteria sedimented from cultures in broth the characteristic absorption spectrum may readily be seen with the spectroscope if a sufficiently intense beam of light is employed. The absorption bands occupy the wave lengths 613-595, 568-5615, 560-551, and 535-512. If the degree of absorption is great, a single broad band may be seen in place of two in the green, from 568 to 555. The number and position of the bands appear to be the same in the various members of the corynebacterium group. No differences have been seen between toxin-producing and non-toxin-producing strains of the species *B. diphtheriae*.

As with the cytochrome observed by Keilin in various materials, the absorption bands of these bacteria fade out when atmospheric oxygen is admitted to the suspension, and reappear when air is excluded or a strong reducing agent (hydrosulphite) is added.

In the examination of filtrates of the corynebacteria the cultures have been grown in the medium, and according to the procedure,

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¹ McMunn, C. A., *Phil. Trans.*, 1886, clxxvii, 267.

² Keilin, D., *Proc. Roy. Soc., London, Series B.*, 1925, xeviii, 312.

³ Yaoi, H., and Tamiya, H., *Sci. Rep. from the Gov't Inst. for Inf. Dis.*, Tokyo, 1927, vi, 85.

employed for the production of diphtheria toxin, after adaptation of the individual strains to growth in a pellicle. After 9 or 10 days of growth the metabolized bouillon was passed through a Berkefeld candle under anaerobic conditions and examined spectroscopically in tubes 200 mm. in length. Observations were made both before and after the addition of a few granules of hydrosulphite to each sample. The results reported here relate to the completely reduced material.

No selective absorption is evident in the sterile unmetabolized bouillon, nor in filtrates from *B. xerosis* and non-toxin-producing strains of *B. diphtheriae*.

Filtrates from toxin-producing strains of *B. diphtheriae* give an absorption spectrum which is well-marked and characteristic and differs in some respects from any absorption spectrum which has been described. A broad band from 555 to 508 with maxima at 536 and 511 corresponds to the absorption in the blue-green by cytochrome and named the "D" band by Keilin. An intense band with maximum at 575 or 576 coincides with one seen in extracts made with strong alkali from yeast by Keilin or by us from the cytochrome-rich *B. phosphorescens*. A band with maximum about 610 corresponds to the "A" band of cytochrome. Another band in the red with maximum between 640 and 630 occupies the position of a band, first observed by Yaoi and Tamiya, in intact cells of *B. dysenteriae* Komagome (but not in Shiga) and in *B. coli*.

Spectrophotometric analyses have shown that a parallel exists between the intensity of spectral absorption and the content of filtrates of various strains in toxin as determined by flocculation tests and animal inoculation. The possibility is, therefore, suggested that diphtheria toxin is derived from cytochrome and that the chemical changes which are involved are similar to those by which a hemochromogen with an absorption at 576 is derived from cytochrome by extraction with alkali.

Just as this work is prepared for publication a note by S. Campbell Smith has appeared in the *Lancet*, March 8, 1930, on the identification of a "porphyrin" in toxic filtrates of *B. diphtheriae*. No relation was observed between the rate of formation of this pigment and the elaboration of toxin. Although the data are meagre, it seems entirely probable that this "porphyrin" is identical with the cytochrome-pigment which we have described.