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Identification of the Porphyrin Compound Found in Cultures of *C. diphtheriæ* and *mycobacteriæ*.

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Porphyrin was found by the present authors[1] in bouillon cultures of *C. diphtheriæ*, in amounts which vary with the toxicity or flocculative titer of the filtrates. Other investigators[2–6] have confirmed the occurrence of this pigment in *C. diphtheriæ* filtrates, by observations made both in the visible and in the ultraviolet spectrum. We have found a pigment, apparently identical, in Old Tuberculin, and in fluid cultures of several species of Mycobacteria. The chemical composition of the pigment, however, has remained in doubt. Its absorption-spectrum is that of a hemochromogen or metal-porphyrin compound. In ethereal extracts sharp bands are found in the visible at 574 and 538 mμ, and in the near ultraviolet an intense absorption with its maximum at 407.9 mμ. The pigment shows an oxidation-reduction change, the oxidized form being colorless. When extracts are treated with strong mineral acid the pigment breaks down, and there appears free coproporphyrin and the copper compound of this substance.

In the effort to identify the pigment we undertook the synthesis* of compounds of coproporphyrin with a number of metallic radicles: Fe, Mg, Mn, Co, Ni, Cu, Sn, and Zn. After an interruption this work has been resumed under more favorable conditions. Of the various metallic compounds studied, only those of Cu, Fe, and Zn are of present significance.

The Zn compound is formed readily when a solution of zinc salt is added to coproporphyrin in slightly alkaline aqueous solution. The combination is stable over a rather wide pH range but is disrupted

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1 Coulter, C. B., and Stone, F. M., *J. Gen. Physiol.*, 1931, **14**, 583.

2 Levaditi, C., Loiseau, G., Païc, M., Phillippe, M., and Haber, P., *Compt. rendu de Soc. Biol.*, 1934, **116**, 609.

3 Wadsworth, A., Crowe, M. O'L., and Smith, L. A., *Brit. J. Exp. Path.*, 1935, **16**, 201.

4 Païc, M., *Compt. rendu de l'Acad. des Scs.*, 1935, **200**, 173.

5 Ottensooser, F., Krupski, A., and Almasy, F., *Biochem. Z.*, 1935, **277**, 314.

6 Pappenheimer, A. M., Jr., and Johnson, S. J., *Brit. J. Exp. Path.*, 1937, **18**, 239.

* A preliminary report of this work was presented at the Conference on Spectroscopy held at M.I.T. on July 18, 1934.

by strong mineral acid. The absorption-spectra in various solvents are very similar. In ethereal solution sharp bands are present about mμ 574 and 539; one of greater intensity is found at mμ 407.8. In aqueous solution the pigment exhibits an oxidation-reduction change, the oxidized form being colorless. The Zn compound thus corresponds in spectrochemical behavior with the pigment contained in culture filtrates. More complete identification is afforded by the correspondence of the fluorescence-spectrum of the pigment from culture filtrates with that of the synthetic compound. Ethereal solutions when excited by light from a carbon arc or other source of intense radiation in the near ultraviolet, give intense bands of emission at mμ 623 and 579. The fluorescence of diphtherial culture filtrates has been described very recently by Dhéré,† whose studies have been pursued independently.

The compounds of the porphyrins with copper are well-known. Copper salts combine readily with coproporphyrin, with greater avidity in acid than in alkaline reaction. The copper compound is formed when an ethereal solution of Zn coproporphyrin is treated with 5% HCl in the presence of even minute traces of Cu. It is insoluble in HCl solution, and is very stable. The intense absorption-band of the Cu compound at Å 3965 betrays its presence by a small peak in the spectrum of culture extracts which give no other evidence of it. Copper thus forms a separate compound in extracts and need not be considered a constituent of the characteristic pigment of culture filtrates. Païc has reached the same conclusion from similar evidence.

Païc has recently ascribed the strong absorption-band at Å 4080 of ethereal extracts to a ferrous compound of coproporphyrin, which he believes to be formed during the extraction of the filtrate with acetic-acid ether. This explanation does not account for the fluorescence, or the visible spectrum of filtrates and ethereal extracts. Neither the ferrous nor the ferric compounds of coproporphyrin show the characteristic absorption-bands of such solutions, nor do they exhibit the phenomenon of fluorescence.

It is not possible at the present time to decide whether Zn coproporphyrin exists within the bacteria, perhaps in combination as a hemochromogen, or is formed in the culture liquid after coproporphyrin has been liberated by the disintegration of bacilli. The latter seems the more likely, but the possibility first mentioned receives support from consideration of the fluorescent emission of living *C. diphtheriæ* cultures, which Dhéré and Rapetti[7] have found

† In press.

[7] Dhéré, Ch., and Rapetti, L., *Extrait du Bull. de l'Acad. de Med.*, 1935,**114**,96.

to occur in the spectral region 619 to 614 mμ. Although no fluorescent band is found within this wavelength range in *ethereal* solution of Zn coproporphyrin, we find the fluorescence bands of alkaline aqueous solution of this compound at mμ 629, 619-613, and 583.5. The most intense of these, with its maximum about 615 mμ, corresponds thus in position with the fluorescent band of the living bacteria.

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Intercellular pH Change Cannot be the Pain Factor in Ischemic Work.

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Using a capillary glass electrode in human extensor digitorum communis muscles carrying an ergograph load of 600 gm. the pH changes between the muscle fibers were determined before and after ischemic work by the method detailed in a previous publication.[1]

With the arm rendered ischemic by a brachial pressure of 160 mm. of mercury work was done until pain appeared. Simultaneously, work was stopped and pressure released. Within 10 seconds pain had disappeared, but maximal intercellular acidity was not reached before 30 to 40 seconds had elapsed. If pH continues to fall after pain ceases intercellular pH fall cannot be the cause of pain.

In another type of trial ischemic work was done until pain appeared, work was stopped and pressure released until acidity reached a plateau. Pressure was then again applied. Intercellular pH again started to fall markedly, far below the previous plateau yet the pain did not reappear. If acidity greater than that present with the pain can be created without pain under the same circumstances, then intercellular pH change cannot be the cause of the pain.

If the sensory endings responsible for ischemic pain lie within the muscle cell pH change might still be the cause of the pain, as there is no proof that intracellular and intercellular pH are identical.

[1] Maison, G. L., Orth, O. S., and Lemmer, K. E., *Am. J. Physiol.*, 1938, **121**, 311.