Short Communication

A Chlorin Separated from the Urine of a Congenitally Porphyric Patient

By T. C. CHU and EDITH J.-H. CHU

Department of Chemistry, Immaculate Heart College, Los Angeles, California, U.S.A.

(Received 12 December 1963)

This is to report the separation of a chlorin from urine samples of a small girl afflicted with congenital porphyria. The samples were supplied by courtesy of Dr Aldrich and Dr Labbe of the University of Washington Hospital, who considered the case as a classical one.

The urine samples were analysed essentially by the method described by Chu & Chu (1962). The average total of urinary porphyrins was 8.5 mg./100 ml., or 62.28 mg./day. No porphobilinogen was detected. On chromatographic separation of the porphyrin esters on a Hyflo column, a green zone was observed above the red uroporphyrin zone. Quantitative estimations of the pigment gave a daily average of about 200 μ g., expressed in terms of porphyrin. After elution in a secondary column with a developing solvent of chloroform (U.S.P.)–light petroleum (b.p. 30–60°) (1:3, v/v), the pigment is pure green in daylight and shows a strong red fluorescence in ultraviolet light. In ethyl acetate it shows two strong absorption maxima at 643 $m\mu$ (2.4) and 495 $m\mu$ (1), and weaker bands near 590 and 540 $m\mu$. The Soret band has a maximum at 393 $m\mu$ (11.8). The relative intensities of the three major bands are given in parentheses. In 5% hydrochloric acid a maximum is found at 621 $m\mu$. Its blue copper complex absorbs at 616 $m\mu$ and the green cobalt complex at 617 $m\mu$, both without fluorescence. The blue zinc complex at 615 $m\mu$ has a red fluorescence. The pigment and its metal complexes have not been obtained in crystalline forms from various solvent mixtures tried.

From the absorption data, the nature of the green pigment as a chlorin is apparent. Herbert (1958) isolated some green porphyrins from porphyric materials and indicated their similarity to the hepatic green porphyrins isolated by Schwartz & Ikeda (1955). Their products absorb at 675 or 708 $m\mu$ and are transformed partly into protoporphyrin on prolonged treatment with acid or alkali, whereas acid removes some of the seven carboxyl groups from this chlorin, yielding unidentified porphyrins in poor yields, as indicated by the modified lutidine paper chromatography (Chu & Chu, 1955). It is neither Barrett's (1956) chlorin a_2 isolated from cytochrome a_2 , nor the green by-product obtained

by Rimington, Mason & Kennard (1958) from their synthetic porphin. The former absorbs at 653 $m\mu$ in ether, whereas the latter absorbs at 630 $m\mu$ in benzene. On the other hand, solutions of porphobilinogen at pH 6 have yielded about 0.5% of this chlorin on standing at room temperature for a week or more, or on brief heating.

The similarity of the absorption spectrum of this chlorin to that of aetiochlorin I (644 and 492 $m\mu$ in ether; Fischer & Orth, 1940) and its formation from porphobilinogen suggest that it is possibly a hepta- or octa-carboxylic chlorin; the paper-chromatographic result favours the heptacarboxylic compound. That the carboxyl groups are located at the side chains of the porphyrin nucleus is evidenced by its characteristic absorption. As an analogy, aetio-, copro- and uro-porphyrins all have the first absorption band in the region of $624 \pm 2 m\mu$.

Further, under the experimental conditions, the photoreduction of porphyrin as reported by Seely & Calvin (1955) would not occur. Therefore the possibility that this chlorin may be an artifact would be ruled out. The 'ill-defined bluish pink fraction, G (iii), with strong absorption at 646 $m\mu$ ' separated from a congenital porphyric patient by Rimington & Miles (1951) might contain some of the chlorin. Whether this chlorin has any special significance in human congenital porphyria remains to be seen.

This work was supported by a grant (AM-01000-12) from the National Institutes of Health, U.S. Public Health Service.

- Barrett, J. (1956). *Biochem. J.* **64**, 626.
 Chu, T. C. & Chu, E. J.-H. (1955). *J. biol. Chem.* **212**, 1.
 Chu, T. C. & Chu, E. J.-H. (1962). *Biochem. J.* **83**, 318.
 Fischer, H. & Orth, H. (1940). *Die Chemie des Pyrrols*, vol. 2, part 2, p. 146. Leipzig: Akademische Verlag G.m.b.H.
 Herbert, F. K. (1958). *Biochem. J.* **69**, 11 p.
 Rimington, C., Mason, S. F. & Kennard, C. (1958). *Spectrochim. Acta*, **12**, 65.
 Rimington, C. & Miles, P. A. (1951). *Biochem. J.* **50**, 202.
 Schwartz, S. & Ikeda, K. (1955). *Ciba Found. Symp.: Porphyrin Biosynthesis and Metabolism*, p. 209. Ed. by Wolstenholme, G. E. W. & Millar, E. C. P. Boston: Little Brown Co.
 Seely, G. R. & Calvin, M. (1955). *J. chem. Phys.* **23**, 1068.