

noted further that crystals were of the typical rosette type, while those of the copro- I ester were the characteristic curved needles which were relatively insoluble in methyl alcohol.

Waldenström⁴ found that uroporphyrin III is extracted by ethyl acetate from an aqueous solution at pH 3.0-3.2 (barely gray-blue to Congo paper by the addition of a very small amount of dilute HCl). The uroporphyrin III from each of the above cases behaved in this manner. It must be emphasized, however, that uroporphyrin is not unique among the porphyrins in being ether insoluble and ethyl acetate soluble, since we have encountered at least one and probably two porphyrins behaving in this manner, but nevertheless clearly distinct from uroporphyrin. These have not yet been identified and are being studied further at the present time.

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A Simple Test for Urinary Porphobilinogen.*

C. J. WATSON AND SAMUEL SCHWARTZ.

*From the Division of Internal Medicine, University of Minnesota Hospital,
Minneapolis, Minn.*

The urines of patients suffering from idiopathic porphyria have often been noted to exhibit strong Ehrlich reactions, in many instances at least, not due to urobilinogen. Waldenström's studies^{1, 2} clearly demonstrated that the chromogen responsible for the Ehrlich reaction in these urines is quite distinct from urobilinogen, which is of course, most often implicated in other pathological states. During the past 3 years we have had opportunity to investigate urine samples from 5 cases of so-called "acute" idiopathic porphyria.[†] In each instance the urine contained the zinc complex of uroporphyrin in considerable amount. The subject of zinc uroporphyrin as a disease entity will be considered in a separate communication. These urines also exhibited Ehrlich reactions in varying degree, at times very

* Aided by a grant from the John and Mary R. Markle Foundation, New York City.

¹ Waldenström, J., Studien über Porphyrie, *Act. Med. Scand. Suppl.*, 1937.

² Waldenström, J., and Vahlquist, B., *Z. f. physiol. Chem.*, 1939, **260**, 189.

[†] We are indebted to Doctors A. R. Hall and John Noble, St. Paul, Minnesota, Doctors C. E. Lynn and A. G. Plankers, Dubuque, Iowa, and Dr. W. H. Ford, Minneapolis, Minnesota, for their permission to study cases 1, 2 and 3 respectively.

intense. In the first 4 cases, the freshly voided urine was already red-brown in color, becoming darker on exposure to light. In the fifth case, the fresh urine was for the most part normal in color, exhibiting a strong Ehrlich reaction. On standing in the light for several days the urine from this case likewise became a deep reddish-brown, containing now both the zinc complex of uroporphyrin and the reddish-brown porphobilin.² Waldenström pointed out that porphobilinogen is much less soluble in organic solvents than is urobilinogen.^{1, 2} In addition to confirming this, we have found that the porphobilinogen aldehyde compound as formed in the Ehrlich reaction is wholly insoluble in chloroform, while that of urobilinogen is readily and easily extracted with this solvent. The reaction as we have carried it out is as follows: Equal parts of urine and Ehrlich's reagent[‡] are mixed in a test tube. To this mixture is then added an equal volume of a saturated aqueous solution of sodium acetate. A few cc of chloroform are then added, and the solutions are thoroughly mixed. The aldehyde compound of porphobilinogen remains in the aqueous fraction while that of urobilinogen is completely extracted by the chloroform. We have repeatedly convinced ourselves that a mixture of the two substances can be separated completely by this method. It should be emphasized that the solution must contain the concentration of salt as achieved by the above proportions; otherwise the extraction of urobilinogen will not be complete. Indol added to the urine exhibits a positive Ehrlich reaction with a color very similar to that given by either urobilinogen or porphobilinogen. The characteristic absorption band is found in the region of 570 m μ (max.) while that of the urobilinogen or porphobilinogen aldehyde compound is maximum at 562 m μ . The indol aldehyde color, like that of urobilinogen, is completely extracted by chloroform under the conditions noted. Thus it is believed that a chloroform insoluble compound, as obtained in the above method, is due to porphobilinogen and is pathognomonic of porphyria. The urine should be examined for this reaction in any case having symptoms suggestive of porphyria, such as unexplained abdominal pain, flaccid paralyses, or other nervous manifestations. Obviously the reaction will be of most value in the cases in which the freshly voided urine is normal in color. The relative insolubility of porphobilinogen in organic solvents has so far prevented purification of the substance, and it is not yet known whether porphobilinogen is also excreted as the zinc complex or whether the zinc is combined only with uroporphyrin.

[‡] 0.7 g p-dimethylaminobenzaldehyde; 150 cc concentrated HCl; 100 cc distilled water.