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Isolation of Crystalline Urobilin from Human Urine.

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Following the isolation of crystalline stercobilin from feces[1, 2] attempts had been made to isolate the corresponding substance, urobilin, from urine. These were unsuccessful. The amalgam reduction of crystalline stercobilin did not yield mesobilirubinogen.[2] Neither did its oxidation with nitric acid yield methyl-ethyl-malein imid which under the same conditions and with the same amounts of substance is easily obtained from mesobilirubinogen. Urobilinogen from urine had already been shown to be identical with mesobilirubinogen, the latter[3] obtained by the amalgam reduction of bilirubin. These facts suggested that stercobilin from feces and urobilin from urine were probably not identical, although very similar. Newer evidence, however, indicates a different explanation of the above findings.

The complete separation of mesobiliviolin and stercobilin, as described in this issue, suggested the application of this principle to extracts of urines rich in urobilin. This has been done with the result that crystalline urobilin was readily obtained. Since both stercobilin and urobilin do not melt sharply, decomposing between 110-130°, other means of identification are necessary. Crystallographic studies as well as measurements of the refractive indices of the two substances are under way. However, their other characteristics including solubilities, absorption spectra, Ehrlich reaction after amalgam reduction, and zinc salts are apparently identical. Preliminary studies of the crystals have shown that both exhibit parallel extinction. Comparative elementary analyses are being carried out.

The crystalline substance was obtained out of several liters of urine rich in urobilin by the following procedure: The urine was strongly acidified with acetic, and shaken out repeatedly with small amounts of chloroform until both fractions no longer showed green fluorescence with zinc acetate. The emulsified portion was shaken with talc and filtered with suction. The chloroform was repeatedly

[1] Watson, C. J., *H. S. Z.*, 1932, **204**, 57.

[2] Watson, C. J., *H. S. Z.*, 1932, **208**, 101.

[3] Fischer, H., and Meyer-Betz, *H. S. Z.*, 1911, **75**, 232.

extracted with distilled water. A considerable portion of the urobilin went into the water, while mesobiliviolin and other impurities remained in the chloroform. The watery solution was made acid with HCl, and the urobilin hydrochloride returned to chloroform. It was returned to 25 HCl, which was diluted to 8% and shaken out again with chloroform. The latter was dried superficially over sodium sulfate, concentrated to a small volume and poured into a large volume of petroleum ether. The precipitated urobilin hydrochloride was redissolved in chloroform and again precipitated with petroleum ether. It was then dissolved in hot chloroform and crystallized on cooling. It was readily recrystallized in the same way.

The urine employed was from a patient with cardiac failure, large liver and ascites. It did not contain bilirubin. The theoretical yield in the above instance was 112 mg. The actual yield of recrystallized urobilin hydrochloride was 15 mg.

Employing the soda-ether-1% HCl-$CHCl_3$ fractionation as for mesobiliviolin and stercobilin, mesobiliviolin, which exhibited the typical absorption spectrum as well as red fluorescence of the zinc salt with absorption at 627 and 583 mm., was obtained from the chloroform fraction of the above after the aqueous extraction. Mesobiliviolin has been observed in the extracts of other urobilin containing urines as well.

Thus it appears that the leukobase of bilirubin (urobilinogen or mesobilirubinogen), which is formed in the bowel, yields at least 2 oxidation products, occurring in the feces and urine, *i. e.,* urobilin or stercobilin and mesobiliviolin.

Preliminary attempts have been made to isolate crystalline urobilin following the *in vitro* oxidation of mesobilirubinogen. A urobilin-like substance regularly occurs along with mesobiliviolin. Employing the above mentioned fractionation, a small crystallization on one occasion took place. The crystals were apparently identical with those of urobilin or stercobilin, and came out of chloroform in the same way. As yet, however, the amount obtained is too small to permit any definite conclusion. This will be repeated using larger amounts of mesobilirubinogen.