between 4 and 18 cm. of water. With peristaltic activity, pressures of 20 to 30 cm. of water were noted.

Pressures were obtained at operation when colostomy was performed for relief of distension in 8 cases of acute colonic obstruction due to malignancy. The sustained pressures varied in these 8 cases between 12 and 52 cm. of water; in 6 of the cases, pressures above 23 cm. of water were recorded, much higher figures than were observed in the small bowel. The duration of obstruction in these cases varied between 3 and 8 days. The significantly greater pressures in colonic obstructions are due to the action of the ileocaecal sphincter[2] which make of the obstructed colon a virtual closed-loop obstruction, in which the intra-enteric pressure may greatly exceed pressures observed in simple obstruction.[3, 4]

8154 P

A Crystalline Hydrobromide of Urobilin (Stercobilin)*

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The identity of crystalline urobilin and stercobilin, as isolated from human urine and feces, was established previously.[1-5] In confirmation of this the same crystalline hydrobromide has been prepared from each substance. The method of preparation is briefly as follows: The free urobilin is prepared from the hydrochloride as previously described.[2] It is not crystallized but is extracted from its chloroform solution with 25% hydrobromic acid. The latter is diluted with 3-4 volumes of distilled water, and the hydrobromide extracted with chloroform. The chloroform solution is dried over anhydrous Na_2SO_4, filtered, and concentrated to a small volume on the water bath. This is poured into at least 10 volumes of petro-

2 Sperling, Louis, *Arch. Surg.*, in press.

3 Burget, G. E., *et al.*, *Arch. Surg.*, 1930, **21**, 829.

4 Morton, J. J., and Sullivan, W. C., *Arch. Surg.*, 1930, **21**, 531.

*Aided by a grant from the University of Minnesota Graduate School.

1 Watson, C. J., *Z. Physiol. Chem.*, 1932, **204**, 57.

2 Watson, C. J., *Z. Physiol. Chem.*, 1932, **208**, 101.

3 Watson, C. J., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1207.

4 Watson, C. J., *Z. Physiol. Chem.*, 1933, **221**, 145.

5 Watson, C. J., *J. Biol. Chem.*, 1933, **105**, 469.

leum ether. After precipitation of the hydrobromide, the precipitate is collected on a small filter paper, and dissolved by repeated extraction with small amounts of hot ethyl acetate. After moderate concentration, the solution is allowed to cool and crystallization soon commences. The yields have varied between 40-50%. The crystals have a very remarkable shape resembling long, narrow boats with several compartments (transverse cleavage lines). The substance is readily recrystallized from ethyl acetate, and has a light yellow color with slight orange cast. M.P. 145-150° (Shrinking above 135°). The absorption spectrum is the same as that of the hydrochloride, and the green fluorescence with zinc acetate appears to be fully as intense. It was conceivable that this substance was a degradation product of urobilin, not simply a hydrobromide. This possibility was excluded by ready conversion to the hydrochloride which was identified by the character of its crystals, and by preparation of its characteristic iron chloride molecular compound.[6]

Heilmeyer and Krebs[7] have recently stated that the hydrochloride of the urobilin which they isolated is a dihydrochloride. In this investigation stercobilin was also isolated by the method which they described. It was found to differ in no way from that isolated according to the method previously described by the writer.[5] From it the crystalline free substance, the iron chloride molecular compound, and the hydrobromide as described above were readily prepared. The hydrobromide contains 12% bromine, a value which agrees very well with formulae for a mono-hydrobromide. Further analyses of the hydrochloride indicate that if the chloroform is carefully removed prior to analysis, the values agree only with a formula for a mono-hydrochloride. In an earlier report the formula $C_{33}H_{44}N_4O_8$ was suggested for the crystalline-free stercobilin. Recent analyses of this, as well as of the hydrochloride and iron chloride molecular compound, which will be reported in detail in a separate publication, have shown that the oxygen content of the molecule is less than at first believed. This is also indicated by the analyses which Heilmeyer and Krebs have reported. Whether 6 or 7 oxygen atoms are contained in the molecule has not yet been determined. Recent analyses of the free substance and of the hydrochloride point to a content of 7; the data for the hydrobromide, however, agree better with 6.

Analytical data:

[6] Watson, C. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 534.

[7] Heilmeyer, L., and Krebs, W., *Z. Physiol. Chem.*, 1934, **228**, 33.

1. Free stercobilin: 3.702 mg. gave 8.84 mg. CO_2, 2.5 mg. H_2O
3.207 " " 0.27 cc. N at 26°, 740 mm.

		C	H	N	O
Theory	($C_{33}H_{46}N_4O_6$)	66.6	7.74	9.43	16.16
	($C_{33}H_{44}N_4O_7$)	64.91	7.54	9.18	18.36
Found		65.1	7.58	9.36	17.96

2. Stercobilin hydrochloride: 3.826 mg. gave 8.60 mg. CO_2, 2.55 mg. H_2O
2.794 " " 0.214 cc. N_2 at 28°, 749 mm.
1.967 " " 0.43 mg. AgCl
3.064 " " 0.695 " AgCl

		C	H	N	Cl
Theory	($C_{33}H_{46}N_4O_6HCl$)	62.85	7.46	8.88	5.55
	($C_{33}H_{46}N_4O_7HCl$)	61.3	7.27	8.66	5.41
Found		61.3	7.45	8.55	5.41
					5.61

3. Stercobilin hydrobromide: 3.950 mg. gave 8.42 mg. CO_2, 2.48 mg. H_2O
2.545 " " 0.199 cc. N_2 at 31.5°, 744 mm.
3.120 " " 0.89 mg. Agbr

		C	H	N	Br
Theory	($C_{33}H_{46}N_4O_6HBr$)	58.65	6.96	8.29	11.85
	($C_{33}H_{46}N_4O_7HBr$)	57.3	6.7	8.1	11.57
Found		58.1	7.02	8.57	12.14

Urobilin hydrobromide (prepared in the same way from crystalline urine urobilin): 3.068 mg. gave 0.240 cc. N_2 at 31°, 744 mm. = 8.59% N.

A detailed report of this study appears elsewhere.[8]

8155 P

Comparison of Natural Crystalline Urobilin (Stercobilin) with that Obtained in Vitro from Mesobilirubinogen.*

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Fischer[1] isolated mesobilirubinogen following an amalgam reduction of bilirubin. Soon afterwards he and Meyer-Betz[2] obtained crystalline urobilinogen from the urine and proved its identity with mesobilirubinogen. There is little doubt that this is the chromogen of natural urobilin or stercobilin, which recent studies[3, 4] have proven identical. It was therefore expected, after allowing crystalline mesobilirubinogen to stand in the air and light until the Ehrlich reaction disappeared and urobilin characteristics had become intense, that a crystalline urobilin could be isolated which

8 Watson, C. J., *Z. Physiol. Chem.*, 1935, **233**, 39.

* Aided by a grant from the University of Minnesota Graduate School.

1 Fischer, H., *Z. Physiol. Chem.*, 1911, **73**, 204.

2 Fischer, H., and Meyer-Betz, *Z. Physiol. Chem.*, 1911, **75**, 232.

3 Watson, C. J., *Z. Physiol. Chem.*, 1933, **221**, 145.

4 Watson, C. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 534.