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.

would be identical with that already obtained from urine and feces. However, it appears that the natural formation of urobilin from urobilinogen is more complex than had been believed. Crystalline mesobilirubinogen (urobilinogen) was isolated from bilirubin according to H. Fischer's method.[1] It was dissolved in glacial acetic acid and remained exposed to the ordinary light and temperature of the laboratory for 3 weeks. After this time the Ehrlich reaction was only very faint. The solution now possessed a dark brown color, exhibited intense urobilin absorption and strong green fluorescence with alcoholic zinc acetate. This solution was poured into 3 volumes of chloroform, and from now on the procedure leading to isolation of the crystalline substance was the same as previously described for isolation of urobilin from urine, and stercobilin from feces.[3, 5] ($CHCl_3$ → H_2O → HCl → $CHCl_3$ → petroleum ether, ppt. → hot $CHCl_3$, crystallization.) In this way a crystalline urobilin has been isolated twice in small amounts. After repeated recrystallization from hot chloroform the melting point of the crystals was not sharp at 155-158°C. Although this substance exhibits intense green fluorescence in alcoholic zinc acetate solution, as well as a "urobilin" spectrum it differs in a number of respects from natural urobilin or stercobilin. The crystals of the hydrochloride have a different appearance in that they are short and thick rectangles, often somewhat barrel shaped, in contrast to the long narrow prisms of the natural material. The substance is obviously much less stabile than the natural urobilin, and this undoubtedly accounts for the poor yields so far obtained (7-12%). An attempt was made to prepare a crystalline iron chloride molecular compound, as well as a hydrobromide, for purposes of comparison, but in both instances the substance underwent definite changes in color, in the first becoming greenish brown, in the second light red. Control experiments with smaller amounts of natural urobilin hydrochloride readily yielded the characteristic crystals of the iron chloride molecular compound and of the hydrobromide.

As yet the amount of "artificial" urobilin isolated has not been enough for microanalysis. This may yield further information as to how the natural and "artificial" urobilins differ. The differences described suggest that some as yet unknown factor is of importance in the natural transition of mesobilirubinogen to urobilin.

A detailed report of this study appears elsewhere.[6]

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[5] Watson, C. J., *J. Biol. Chem.*, 1934, **105**, 469.

[6] Watson, C. J., *Z. Physiol. Chem.*, 1935, **233**, 39.