150 cc. of 2% solution of sodium chloride at 2°C. for 48 hours. The liquid was decanted, centrifuged, and mixed with an equal volume of saturated solution of ammonium sulfate. The "globulin" was recovered by centrifuging and filtering on hardened paper, dissolved in 2% sodium chloride, and dialyzed until free from sulfate and chloride ions. The precipitate was then recovered on hardened paper and oven-dried. From 40 placentas a yield of 10.5 gm. of globulin resulted; it contained 12.73% nitrogen and 0.61% ash.

The nitrogen-distribution was determined by the method of Van Slyke. The values reported are the averages of duplicate determinations calculated as percentages of the total nitrogen. Some values reported in the literature are given for comparison.

The distribution of nitrogen in placental globulin is essentially that found by Cavett[3] and Widdowson[7] in human serum-globulin, and very similar to the values reported by Banzhaf, Suguira and Falk[2] for normal serum-globulin; also to the values for the globulins of normal and colostral milks as reported by Crowther and Raistrick[8] and those reported by Hartley[5] for animal serum-globulins. This comparison is based largely upon the values for ammonia and arginine which are determined directly and with reasonable accuracy. With the exception of cystine, which is present in smaller quantities, the remaining bases also agree closely. The analysis of whole placental tissue, however, presents an entirely different picture due to other constituents.

8629 P

Fate of Parenterally Administered Crystalline Urobilin; Urobilin Tolerance Test of Liver Function.

CECIL JAMES WATSON. (Introduced by H. A. Reimann.)

From the Department of Medicine, University Hospitals, Minneapolis.

Crystalline urobilin, as isolated from feces by the writer's method,[1, 2] may be administered intramuscularly or intravenously

3 Private communication, Cavett, J. W.

5 Hartley, P., *Biochem. J.*, 1914, **8**, 541.

7 Widdowson, E. M., *Biochem. J.*, 1933, **27**, 1321.

8 Crowther, C., and Raistrick, H., *Biochem. J.*, 1918, **10**, 473.

1 Watson, C. J., *J. Biol. Chem.*, 1934, **105**, 469.

2 Watson, C. J., *Z. physiol. Chem.*, 1935, **233**, 39.

without untoward reaction. In the present investigation it was customary to dissolve 50 mg. of the recrystallized hydrochloride in 2 cc. of 95% alcohol. After dilution with 8 cc. of physiological saline containing .1 gm. of sodium bicarbonate the resultant solution was sterilized in the autoclave for 15 minutes at 15 lb. pressure. Recovery experiments have shown that the substance is not damaged by this treatment.

Urobilin does not give an Ehrlich reaction; consequently, it was surprising to find, after parenteral injection of the above mentioned solutions, that the Ehrlich reaction of the urine regularly became positive or intensified within one-half to 2 hours. It was evident that the substance had become reduced somewhere in passing from muscle or blood stream to the urine. The possibility had to be considered that the added load on the liver occasioned by a sudden addition of 50 mg. of urobilin to the circulation had caused it to refuse native urobilinogen coming from the bowel. This possibility was excluded by administration of crystalline urobilin to an individual with complete neoplastic common duct obstruction, whose urine and feces contained no urobilinogen. Following an injection of 50 mg., either intramuscularly or intravenously, the Ehrlich reaction of the urine became strongly positive within one to 2 hours. Studies are now in progress to determine the site of this reduction in the body. It is evident that all of the injected substance is not reduced, since unchanged urobilin may be demonstrated spectroscopically in the fresh urines obtained one-half to 4 hours after its administration.

In the normal individual, following intramuscular injection of 50 mg. of crystalline urobilin, the fraction appearing in the urine is relatively small. Both in the normal and in individuals with liver disease, except where renal insufficiency is also present, all or nearly all of this fraction appears within 6 to 8 hours after injection. However, it is more reliable to compare the 12 or 24-hour urobilinogen excretion preceding injection with that for a similar period immediately thereafter. As yet studies have been limited to individuals whose 24-hour urine urobilinogen was normal (0-4 mg.) or not more than slightly elevated. The method used depends upon the Ehrlich reaction and is described elsewhere.[3] The data in Table I suggest that the amount excreted in the urine following injection of 50 mg. of urobilin, may quite possibly be correlated with the functional state of the liver.

Because of normal variation in urobilinogen content of the

[3] Watson, C. J., *Am. J. Clin. Path.*, in press.

TABLE I.

Diagnosis	How given	Urine Urobilinogen Before injection	Urine Urobilinogen After injection	Period of Collection hr.
1. Normal	a. Intramuscularly	Trace	2.01	24
	b. Intravenously	"	5.04	24
2. Normal (previous catarrhal jaundice; mild alcoholism)	Intramuscularly	2.6	4.6	24
3. Normal	"	Trace	2.1	24
4. Normal (quiescent gastric ulcer)	"	0	2.6	12
5. Probable cirrhosis of the liver. (Previous jaundice; enlarged spleen, bleeding varices.)	a. "	2.4	11.5	12
	b. Intravenously	2.6	32.0	12
6. Complete common duct obstruction due to metastatic carcinoma; (hard, nodular liver).	a. Intramuscularly	0	12.3	24
	b. Intravenously	0	22.3	24
7. Hepar lobatum (ascites, no jaundice)	Intramuscularly	5.3	10.1	24
8. Recent neo-arsphenamine icterus; cystotomy, pyelonephritis, renal insufficiency.*	a. Intramuscularly	5.1	10.5	24
	b. Intravenously	5.5	11.2	24

* In this instance excretion of the injected urobilin was obviously slowed since the qualitative tests did not become positive shortly after injection as is usually true.

feces,[4] a single injection of 50 mg. of urobilin would be insufficient to determine the fraction lost in the feces. The following experiment indicates that this fraction is relatively small.

460 mg. of crystalline urobilin were given intramuscularly, in divided doses not exceeding 50 mg. each, during the first 6 days of an 8-day period in which the total amount of urobilinogen in the feces was 400 mg. Based on estimations made during periods of similar length before and afterwards, the expected excretion, apart from the administered urobilin, was 268 mg.† The difference of 132 mg., or 29% of the injected amount, may be considered to represent the fraction reappearing in the feces. Since the amounts in the urine were negligible, it seems evident that the majority of the injected substance was retained.

[4] Watson, C. J., *Arch. Int. Med.*, in press.

† The subject of this study suffered from a mild idiopathic hypochromic anemia; a disease characterized by reduced urobilinogen excretion.[4]