

Intestinal Absorption of Bile Pigments. III. The Enterohepatic Circulation of Urobilinogen in the Rat *

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Bile pigment excretion is completed in the intestine. To maintain the efficiency of the excretory process, mechanisms must be available that prevent or limit intestinal absorption of pigment and pigment derivatives. Conjugation of bilirubin with glucuronic acid appears to serve this purpose. Although unconjugated bilirubin is readily absorbed from both the small and large bowel, absorption of the water-soluble glucuronide is minimal (1-3). Since essentially all bilirubin is conjugated before its excretion into the bile (4, 5) and since the conjugate probably remains intact until it reaches the terminal ileum (2, 3), pigment is transported efficiently through the small bowel with reabsorption held to a minimum.

The bacteria of the terminal ileum and large bowel reduce conjugated bilirubin to a series of unstable chromogens—stercobilinogen, mesobilirubinogen, and *d*-urobilinogen—which collectively are termed urobilinogen (6). It is a widely held view that urobilinogen is in part absorbed from the large bowel and reexcreted in the bile and, to a lesser extent, in the urine (6, 7a). Direct experimental verification of this concept has not been possible heretofore largely because of limitations inherent in the standard method for identifying and quantitating urobilinogen. Although microgram quantities of urobilinogen can be estimated by color formation with *p*-dimethylaminobenzaldehyde (8, 9), the technique is nonspecific (10), the urobilinogen-aldehyde complex is unstable (11), and the coupling reaction is inhibited by bio-

logic materials and common laboratory reagents (12, 13).

These difficulties have now been overcome by preparation of a radioactive chromogen of the urobilinogen group, mesobilirubinogen-C¹⁴. After its radiochemical purity was established, the labeled chromogen was used to investigate the intestinal absorption of urobilinogen in rats (14). Comparable investigations in man will be reported in separate communications (15, 16).

Methods

Preparation of the radioactive chromogen. Crystalline unconjugated bilirubin-C¹⁴ prepared biosynthetically (17) was reduced to mesobilirubinogen-C¹⁴ (Figure 1) by the method of Fischer (18). Starting materials consisting of 30 to 65 mg of radioactive pigment, 4 g of 3% sodium amalgam (19), and 2 ml of 0.1 N sodium hydroxide were shaken vigorously for 1 hour in a tightly stoppered glass vial. After dilution with 30 ml of distilled water and acidification to pH 6 with glacial acetic acid, the reaction mixture was extracted repeatedly with 200 ml of a solution consisting of 20 parts petroleum ether (bp 30° to 60° C) and one part of peroxide-free ethyl ether. The combined ether extracts were washed twice with water and then taken to dryness *in vacuo*, which yielded a colorless amorphous powder. The residue was dissolved in hot ethyl acetate, and crystals of mesobilirubinogen-C¹⁴ formed on cooling at 4° C for 12 hours. Because of the extreme lability of the chromogen, each of these procedures was performed in subdued light, and the crystals were stored in high vacuum and in the dark until immediately before use.

Assay of radiochemical purity. The specific activity of the chromogen was determined by dissolving weighed amounts of mesobilirubinogen-C¹⁴ in chloroform and counting measured portions of these solutions in a Packard Tri-Carb liquid scintillation spectrometer (17). The efficiency of counting was determined through the use of a toluene-C¹⁴ internal standard, and the results could therefore be expressed as disintegrations per minute per microgram of mesobilirubinogen-C¹⁴.

The crystalline material coupled with *p*-dimethylaminobenzaldehyde (Ehrlich reagent), forming the characteristic purple urobilinogen-aldehyde complex (8, 9). Its

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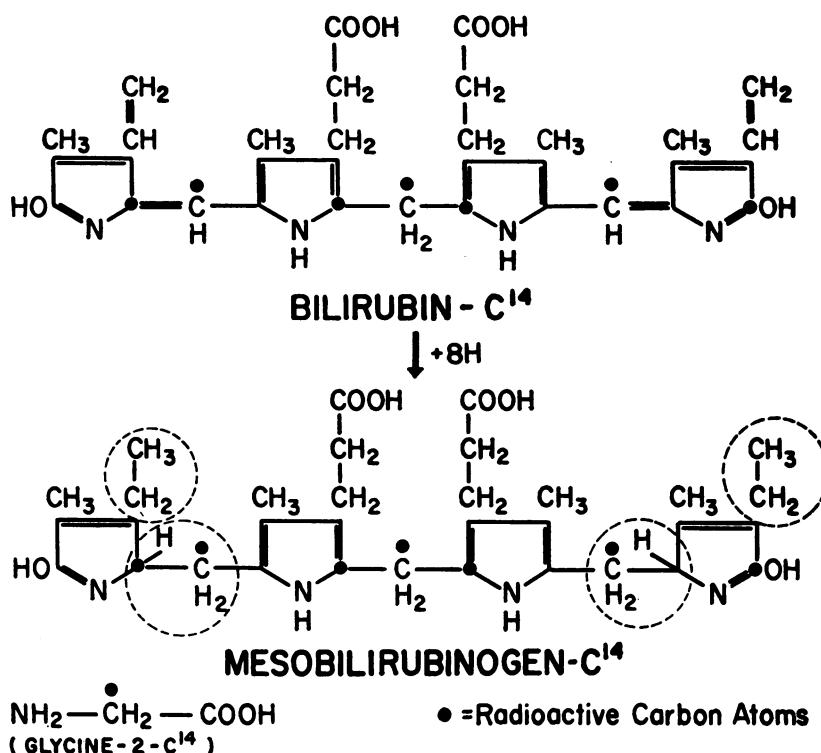


FIG. 1. SCHEMATIC REPRESENTATION OF REDUCTION OF BILIRUBIN-C¹⁴ TO MESOBILIRUBINOGEN-C¹⁴. Bilirubin-C¹⁴ was obtained biosynthetically, with glycine-2-C¹⁴ as the starting material.

absorption spectrum in the visible range was determined spectrophotometrically, and optical density at peak absorbance was compared with previously established values, using a pontacyl dye standard (8, 9). In addition, radioactive chromogen dissolved in petroleum ether was oxidized with an aqueous iodine solution (20). The resulting pigment was extracted from the aqueous phase into chloroform and crystallized and recrystallized from methanol-ethyl acetate. Spectrophotometric determinations were performed on crystals dissolved in methanol containing 3% (vol/vol) hydrochloric acid or 5% (wt/vol) zinc acetate (Schlesinger reagent).

Mesobilirubinogen-C¹⁴ dissolved in methanol was applied in 5- μ l samples to dry 1-mm thick layers of silica gel G adhering to 5- \times 20-cm glass plates. Ascending chromatograms were developed in the following systems: system I, phenol/methanol/water (15/5/1); system II, methanol/water/pyridine (32/8/3.2); system III, *n*-butanol/water/pyridine (1/1/1). After drying, the mesobilirubinogen-C¹⁴ was located by spraying the chromatogram with *p*-dimethylaminobenzaldehyde and saturated sodium acetate. The sprayed chromatogram was then divided into consecutive sections and scraped into low-potassium glass counting vials¹ containing 1 ml of M Hyamine. The suspension of silica gel in Hyamine was

stirred continuously with a glass rod for 5 minutes, after which scintillator fluid was added and radioassay was performed (21). Radioactivity (disintegrations per minute) in individual sections of the chromatogram was expressed as a per cent of the total isotope on the chromatogram.

The melting point of the chromogen crystals was determined in triplicate with a modified Johns melting point apparatus² previously calibrated with crystalline L-ascorbic acid.

Preparation of experimental animals and administration of mesobilirubinogen-C¹⁴. Fasting male Sprague-Dawley rats weighing 300 to 400 g were anesthetized with Nembutal and ether and prepared with an external biliary fistula and with a plastic square sutured around the anus (2). Thereafter, with the laparotomy wound open, the radioactive chromogen was administered intravenously, infused by peroral duodenal tube, or injected directly into the terminal ileum. The wound was then closed, and the animals were placed in a modified Bollman restraining cage and permitted to recover from anesthesia. Bile and urine specimens were collected separately and quantitatively (2) and analyzed as described below.

Two rats were injected intravenously with mesobilirubinogen-C¹⁴ dissolved in rat serum (Table I, rats no.

¹ Packard Instrument Co., La Grange, Ill.

² Fisher Scientific Co., Chicago, Ill.

TABLE I
Intestinal absorption and excretion of mesobilirubinogen-C¹⁴

Rat no.	Wt	Duration of experiment	Route of administration	Characteristics of administered material			Radioactivity recovered*	
				Volume	Mesobilirubinogen-C ¹⁴	Radioactivity	Bile	Urine
	g	hrs		ml	μg	dpm	%	%
1	360	4	Intravenous	4.5	1,950	132,700	92	4
2	340	4	Intravenous	4.8	1,780	121,200	85	<1
3	400	4	Intravenous†	1.4	1,910	196,600	90	3
4	350	48	Into duodenum	5.2	4,080	277,800	57	1
5	330	72	Into duodenum	6.1	3,770	452,500	65	3
6	360	48	Into duodenum	0.7	670	62,200	64	1
7	320	48	Into duodenum	2.2	880	11,000	57	<1
8	360	24	Into duodenum‡	2.5	3,790	443,200	82	<1
9	320	24	Into duodenum§	2.3	2,390	221,600	68	1
10	300	72	Into terminal ileum	5.7	3,560	241,900	9	<1
11	310	72	Into terminal ileum	6.1	2,820	438,800	21	2
12	380	48	Into terminal ileum	3.0	861	137,700	7	3
13	400	4.5	Intravenous	3.4	1,790	180,700	77	3
14	390	48	Into duodenum	2.5	3,060	319,000	67	4
15	350	24	Into duodenum¶	1.8	2,920	303,500		99
16	310	24	Into duodenum¶	2.8	2,880	266,800		64

* Percentage of administered radioactivity.

† Homozygous male Gunn rat.

‡ Bile obtained from rat that received intravenous mesobilirubinogen-C¹⁴.

§ Bile obtained from rat that received intraduodenal mesobilirubinogen-C¹⁴.

|| Rat pretreated with carbon tetrachloride.

¶ Occlusion of common bile duct.

1, 2). An additional comparable study was performed on a mutant Wistar (Gunn) rat with hereditary non-hemolytic hyperbilirubinemia (22) due to deficiency of hepatic glucuronyl transferase (23) (Table I, rat no. 3). In four rats radioactive chromogen dissolved in 7 mM aqueous taurocholate was infused by peroral tube into the duodenum (Table I, rats no. 4 to 7), and in three other animals by direct injection into the terminal ileum (Table I, rats no. 10 to 12). Two rats received duodenal infusions of rat bile containing mesobilirubinogen-C¹⁴ (Table I, rats no. 8, 9). These bile specimens had been collected previously from animals that had received radioactive chromogen intravenously or by the intraduodenal route.

Two rats were pretreated with carbon tetrachloride, 0.25 ml injected subcutaneously three times per week until a total of 7 ml had been administered; mesobilirubinogen-C¹⁴ was then given intravenously to one and intraduodenally to the other animal (Table I, rats no. 13, 14). At the conclusion of these experiments, the livers were examined histologically to assess the extent of chronic hepatic injury.

Finally, in two rats, the common bile duct was occluded with three silk ligatures and then transected at two points between the ties (Table I, rats no. 15, 16). Mesobilirubinogen-C¹⁴ was infused into the duodenum, and the animals were caged for quantitative collection of the urine.

Radioassay and analysis of the excreta. Radioassay of bile and urine was performed in a Packard Tri-Carb liquid scintillation spectrometer by techniques described previously (2). Urobilinogen concentration in bile was determined spectrophotometrically by complex formation with *p*-dimethylaminobenzaldehyde (8, 9). Samples of

bile were adjusted to pH 6 with acetate buffer and extracted three times with 2 vol of a 9:1 mixture of petroleum ether (bp 30° to 60° C) and peroxide-free ethyl ether. The concentration of urobilinogen (8, 9) and of radioactivity (17) in the ether phase was determined. Wherever possible, chemical manipulations and assay procedures were performed rapidly, in subdued light, and at 4° C to prevent or minimize deterioration of the unstable mesobilirubinogen.

To exclude the possibility that, in the course of absorption and reexcretion, mesobilirubinogen-C¹⁴ was converted to bilirubin-C¹⁴ (7b), bilirubin from bile samples was isolated and subjected to radioassay (17).

Results

Preparation of mesobilirubinogen-C¹⁴. On reduction of bilirubin-C¹⁴, amorphous mesobilirubinogen-C¹⁴ was obtained in yields ranging from 40 to 60%. Approximately one-tenth of this crude preparation could be recovered in crystalline form from hot ethyl acetate. Within the sensitivity range of the techniques employed, amorphous, crystalline, and recrystallized mesobilirubinogen-C¹⁴ exhibited the same specific activity. The chromogen remained colorless for 2 to 3 weeks when stored in a high vacuum and in the dark. Even under optimal storage conditions, however, evidence of deterioration began to appear after 3 weeks, necessitating the preparation of fresh lots of mesobilirubinogen-C¹⁴ at frequent intervals. In

addition to being exceedingly labile, the chromogen was intensely hygroscopic and readily developed an electrostatic charge. These characteristics complicated all transfer procedures.

Mesobilirubinogen- C^{14} coupled with *p*-dimethylaminobenzaldehyde to form the characteristic purple complex with maximal absorption at 556 $m\mu$ (24). The optical density at peak absorption corresponded, weight for weight, to previously reported values for mesobilirubinogen and for a pontacyl dye standard (8, 9). On exposure to iodine, the labeled chromogen was oxidized to an orange-red pigment, which was crystallized by displacement of methanol with boiling ethyl acetate. The pigment emitted intense green fluorescence when dissolved in Schlesinger reagent and exposed to ultraviolet light (25). The sharp absorption band at 493 $m\mu$ obtained by dissolving the pigment in 3% hydrochloric acid in methanol, and the corresponding band at 509 $m\mu$ in methanolic zinc acetate, were characteristic of urobilin (26a).

Thin-layer chromatography yielded the following R_f values for mesobilirubinogen- C^{14} : system I, 0.52; system II, 0.81; system III, 0.89. Despite the instability of the chromogen when dissolved

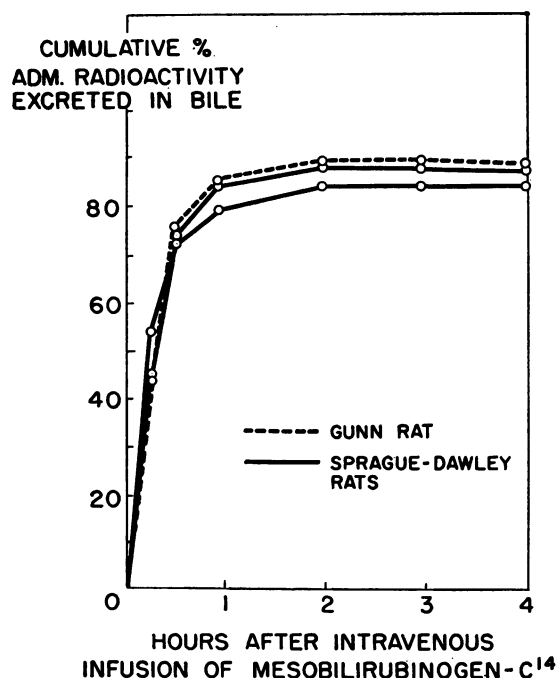


FIG. 2. RADIOACTIVITY IN BILE AFTER INTRAVENOUS ADMINISTRATION OF MESOBILIRUBINOGEN- C^{14} .

TABLE II
Radioactivity excreted in bile in the form of intact mesobilirubinogen- C^{14}

Rat no.	Route of administration	Mesobilirubinogen- C^{14} in bile*
		%
1	Intravenous	98
2	Intravenous	100
3	Intravenous†	100
4	Into duodenum	68
5	Into duodenum	82
6	Into duodenum	89
7	Into duodenum	100
8	Into duodenum‡	96
9	Into duodenum§	100
10	Into terminal ileum	
11	Into terminal ileum	
12	Into terminal ileum	
13	Intravenous¶	100
14	Into duodenum¶	60

* Radioactivity in the form of mesobilirubinogen- C^{14} expressed as percentage of total radioactivity in bile specimen.

† Homozygous male Gunn rat.

‡ Bile obtained from rat that received intravenous mesobilirubinogen- C^{14} .

§ Bile obtained from rat that received intraduodenal mesobilirubinogen- C^{14} .

|| Mesobilirubinogen- C^{14} in individual bile samples too low to obtain accurate Ehrlich determinations.

¶ Rat pretreated with carbon tetrachloride.

in these three solvents, 90 to 96% of the isotope on the developed chromatogram was contained within the *p*-dimethylaminobenzaldehyde-positive area.

The melting point of the mesobilirubinogen- C^{14} crystals equaled 198° C, which agrees with the range of 197° to 202° C reported in the literature (26b).

Mesobilirubinogen- C^{14} excretion after intravenous administration. Biliary excretion of isotope began during the first 15 minutes after intravenous injection of mesobilirubinogen- C^{14} and was nearly complete at the end of 1 hour (Figure 2). Eighty-five to 92% of the administered label appeared in the bile during the 4 hours of observation, whereas over the same period less than 5% of the isotope was excreted in the urine (Table I, rats no. 1, 2). Similar results were obtained in the congenitally icteric Gunn rat (Figure 2; Table I, rat no. 3). To estimate the fraction of radioactivity excreted in the bile in the form of mesobilirubinogen- C^{14} , the specific activity of the chromogen appearing in the bile (disintegrations per minute per microgram as estimated by the Ehrlich reaction) was compared with that of the injected

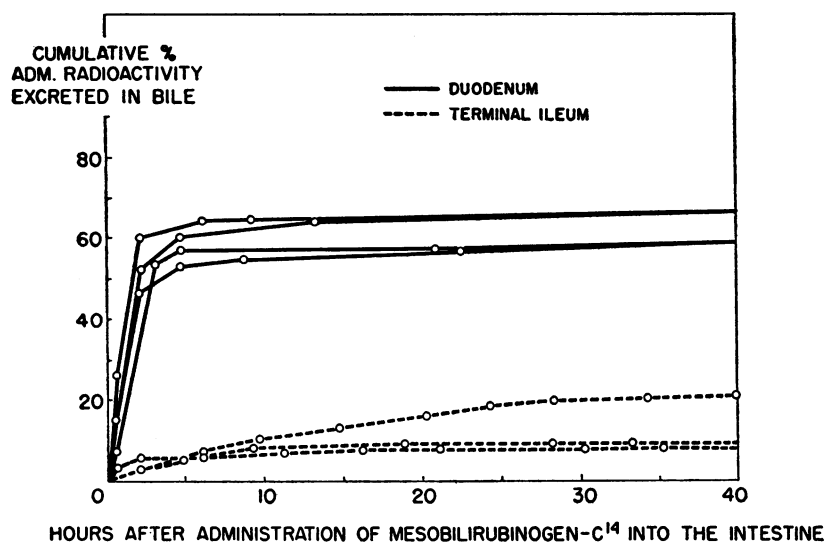


FIG. 3. RADIOACTIVITY IN BILE AFTER ADMINISTRATION OF MESOBILIRUBINOGEN- C^{14} INTO THE INTESTINE.

material. It was found that 98 to 100% of the radioactivity present in the bile was excreted in the form of the intact radioactive chromogen (Table II). Moreover, the greater proportion of the mesobilirubinogen in the bile could be extracted into the highly nonpolar solvent mixture of petroleum ether and ethyl ether (9:1).

Intestinal absorption of mesobilirubinogen- C^{14} . Absorption and biliary reexcretion of the label began promptly after infusion of mesobilirubinogen- C^{14} into the intestine, reaching maximal rates during the first 2 hours and nearing completion within 12 hours (Figure 3). After intraduodenal administration, total biliary excretion equaled 57 to 65% of the dose, but when the chromogen was injected into the terminal ileum, the magnitude of absorption and reexcretion was reduced to 7 to 21% (Table I). Urinary excretion of radioactivity was less than 5% of the administered dose (Table I) in all experiments.

In rats no. 4 to 9 (Tables I and II), 68 to 100% of the radioactivity in the bile was present in the form of intact mesobilirubinogen- C^{14} . In each instance a major fraction of the labeled chromogen could be extracted from the bile with a mixture of petroleum ether and ethyl ether (9:1). In several experiments, the ether extracts were oxidized with iodine, and crystalline urobilin- C^{14} was obtained by the technique described above; the

specific activity of the isolated pigment was comparable to that of the chromogen in the bile. The amounts of chromogen absorbed and reexcreted after injection into the terminal ileum remained below the level that could be identified accurately by direct examination of bile with Ehrlich reagent (Tables I and II, rats no. 10 to 12). A confirmatory study was performed, therefore, in which the dose of mesobilirubinogen injected into the terminal ileum was increased to 6 mg. Ten per cent of the administered material was absorbed and reexcreted in the bile in the form of Ehrlich-positive chromogen.

There was no evidence to suggest that mesobilirubinogen- C^{14} could be converted to bilirubin- C^{14} , regardless of the site of chromogen administration.

In the two animals that received intraduodenal infusions of rat bile containing mesobilirubinogen- C^{14} (Table I, rats no. 8, 9), the results were comparable to those obtained with the radioactive chromogen dissolved directly in taurocholate. Biliary excretion of the label began promptly and ultimately totaled 82 and 68% of the administered dose; intact chromogen was identified in the bile by the Ehrlich reaction (Table II).

Absorption and excretion of mesobilirubinogen- C^{14} in the presence of liver damage or biliary obstruction. Because it had previously been sug-

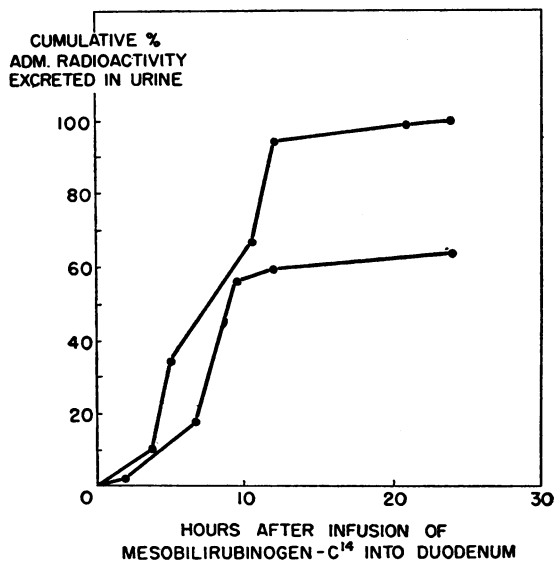


FIG. 4. RADIOACTIVITY IN URINE AFTER ADMINISTRATION OF MESOBILIRUBINOGEN- C^{14} INTO DUODENUM OF TWO RATS WITH COMPLETE BILIARY OBSTRUCTION.

gested that "urobilinogen tolerance" might provide a sensitive index for the detection of liver injury (27), studies were performed in rats with carbon tetrachloride-induced hepatic damage. Despite the presence on histologic sections of moderate fatty infiltration of the liver and moderate disruption of the hepatic architecture, 77% of the mesobilirubinogen- C^{14} administered by intravenous injection appeared in the bile within 2 hours (Table I, rat no. 13). Similarly, in another instance (Table I, rat no. 14) when radioactive chromogen was infused into the duodenum, 67% was absorbed and reexcreted in the bile. Urinary excretion of the label did not exceed 5% in either experiment.

The magnitude of mesobilirubinogen- C^{14} absorption from the duodenum of the two rats with complete biliary obstruction (Table I, rats no. 15, 16) was comparable to that in unobstructed animals. However, the label appeared in the urine instead of in the bile, and the rate of excretion was slower and less uniform (Figure 4).

Discussion

Investigators have been intrigued for decades with the possibility that bilirubin and its major end product, urobilinogen, are absorbed from the intestine and reexcreted by the liver (6, 7). The occurrence of a significant "enterohepatic cir-

lation" of bile pigment or pigment derivatives would indicate that the intestinal phase of heme catabolite excretion is inefficient. Moreover, in the presence of compromised hepatic function, this increase in the excretory load might significantly influence the development of hyperbilirubinemia.

Previous investigations performed in this laboratory have defined the mechanisms that normally prevent intestinal absorption of bilirubin (1-3). The following points were established: 1) Unconjugated bilirubin is chloroform soluble, but virtually insoluble in aqueous solution at physiologic pH. 2) The pigment is readily absorbed and reexcreted by the liver (1-3) when introduced experimentally into the intestine; absorption probably occurs by passive diffusion across the intestinal mucosa, which is permeable to many organic molecules of comparable size and solubility characteristics (28). 3) Under physiologic conditions essentially all bilirubin excreted into the intestine is conjugated with glucuronic acid (4, 5), and the conjugate probably remains intact during transit through the small bowel (2, 3). 4) Bilirubin glucuronide is highly polar and of greater molecular weight than unconjugated bilirubin; as would be anticipated from these physical properties (28), the absorption of intact conjugate is below the level of measurability.

It is apparent from these observations that under physiologic conditions, little intestinal absorption of intact bilirubin occurs. On the other hand, the existence of a significant enterohepatic circulation of the main bilirubin derivative, urobilinogen, is widely (6, 7) although not universally (29) accepted. Antibiotics that prevent the conversion of bilirubin to urobilinogen in the large bowel eliminate or greatly decrease urinary excretion of urobilinogen (30). In experiments with dogs, it was found that urobilinogen disappeared from freshly excreted bile when external biliary drainage eliminated intestinal formation of urobilinogen (31). Several investigations performed with unlabeled chromogen suggested the occurrence of intestinal absorption of urobilinogen (32-34). The preparation of mesobilirubinogen- C^{14} described in this study has provided the opportunity for direct demonstration of intestinal urobilinogen absorption in the rat and has permitted evaluation of the magnitude of this process.

In the various tests performed, the C¹⁴-labeled chromogen preparations appeared to exhibit a high degree of radiochemical purity. The specific activity remained constant during successive crystallizations; melting point determinations fell within established values; the spectroscopic properties of the urobilinogen-aldehyde complex and of the oxidation product of the chromogen, urobilin, matched data previously reported; despite known instability in the solvent systems employed, 90 to 96% of radioactivity migrated with the *p*-dimethylaminobenzaldehyde-positive material on thin-layer chromatography.

As in previous investigations of intestinal bilirubin absorption (2), certain limitations were inherent in the experimental technique employed, and these should be noted. All studies were performed in partially restrained animals in a post-operative state. The radioactive chromogen was infused into the intestinal lumen in a single pulse rather than by the continuous accumulation that occurs under physiologic conditions. In most of the studies, the dose of mesobilirubinogen exceeded the anticipated daily urobilinogen formation in the rat (23, 35), but in a few instances, the amounts employed were within the range of the animal's daily bile pigment production (Table I, rats no. 6, 7, 12); the results in these two groups of experiments were entirely comparable. It should be noted further that the physiologic behavior of mesobilirubinogen may not be representative of that of the other members of the urobilinogen group. Finally, for purposes of comparison, a series of experiments was carried out in which mesobilirubinogen was infused into the duodenum, although formation of urobilinogen occurs only in the terminal ileum and in the large bowel (6) under physiologic conditions.

Within these limitations, it has been demonstrated that urobilinogen is absorbed along the entire length of the digestive tube and is reexcreted predominantly in the bile. This finding is comparable to that obtained with unconjugated bilirubin (2) and was predictable in view of the solubility properties of the chromogen. Mesobilirubinogen dissolves sparingly in water at physiologic pH and maximally in chloroform among common laboratory organic solvents. It therefore should transfer readily across the lipid membranes of the intestinal mucosa (28). More

than half of the chromogen dose infused into the duodenum was absorbed (Table I) and reexcreted rapidly in the bile (Figure 3). When the labeled mesobilirubinogen was administered into the terminal ileum, on the other hand, both the rate and the total magnitude of absorption and reexcretion were diminished (Table I, Figure 3). The demonstrated difference in absorption may be attributable to the lesser absorptive surface of the large bowel, to differences in the absorptive function of individual cells lining the upper and lower portions of the intestine, or to adsorption of chromogen on unabsorbable fecal material. Moreover, it has been suggested that bilirubin glucuronide may in part be reduced to urobilinogen without concomitant splitting of the ester glucuronide bond (6). Unfortunately, conjugated urobilinogen has never been prepared, and experimental study of its intestinal absorption cannot at present be performed. It is predictable, however, from data obtained in studies with bilirubin glucuronide, that persistence of the conjugate bond would greatly reduce absorption of the chromogen. In view of this consideration and on the basis of the present results, it is probable that a small but definite enterohepatic circulation of urobilinogen occurs under physiologic conditions.

Under the experimental conditions, a major fraction of the mesobilirubinogen-C¹⁴ absorbed and reexcreted in the bile reacted with *p*-dimethylaminobenzaldehyde and could be extracted with a nonpolar solvent mixture. Furthermore, significant quantities of labeled urobilinogen excreted in the bile of a rat were absorbed and reexcreted when the bile specimens were infused into the duodenum of two other animals (Table I, rats no. 8, 9). Finally, after intravenous administration, the Gunn rat excreted mesobilirubinogen-C¹⁴ at a normal rate (Figure 2). These observations provide suggestive but not conclusive evidence that a major fraction of the urobilinogen is absorbed and reexcreted without conjugation to glucuronic acid or other polar molecules. This does not exclude the possibility that during the process of intestinal absorption and reexcretion, a minor portion of the urobilinogen may undergo chemical alterations. There is no evidence, however, for reoxidation of mesobilirubinogen to bilirubin.

Urine normally contains small amounts of urobilinogen, but in the two rats with complete ob-

struction of the common bile duct, all absorbed mesobilirubinogen-C¹⁴ was excreted by the kidneys (Table I, rats no. 15, 16; Figure 4). Although there was no significant urinary spillover in the two rats with relatively mild hepatic injury (Table I, rats no. 13, 14), studies performed in humans have suggested that increased urinary excretion of absorbed mesobilirubinogen-C¹⁴ may occur as a result of modest reductions in hepatic function (15, 16). Urobilinogenuria has long been recognized as a laboratory finding in some patients with diseases of the liver and biliary tree (36). The present findings are consistent with the view that this phenomenon may result from impaired hepatic excretion of chromogen absorbed from the large bowel. In addition, however, the presence of liver disease may permit invasion of the small bowel by the fecal microflora (37), and under these conditions urobilinogen formation may take place in the more proximal portions of the intestine. Similar bacterial invasion occurs in diseases or experimentally produced abnormalities of the small bowel associated with significant intestinal stasis (38, 39). Since mesobilirubinogen-C¹⁴ absorption is more rapid and complete from the small bowel, as compared to the large, it is possible that in these instances, urobilinogenuria reflects enhanced intestinal chromogen absorption as well as impaired hepatic excretion.

Summary

1. A method is described for the preparation of crystalline mesobilirubinogen-C¹⁴ by sodium amalgam reduction of bilirubin-C¹⁴. Radiochemical purity of the chromogen was established by spectroscopic and chromatographic means, by melting point determination, and by recrystallization to constant specific activity.

2. After intravenous injection of mesobilirubinogen-C¹⁴ into rats, 85 to 92% of the dose was excreted in the bile within 4 hours. When labeled chromogen was infused into the intestine, a significant fraction was absorbed intact and re-excreted in the bile, with insignificant amounts of isotope appearing in the urine. The rate and magnitude of absorption from the small bowel were much larger than the values obtained when labeled chromogen was injected into the large bowel. In the presence of complete biliary ob-

struction, the absorbed mesobilirubinogen-C¹⁴ was excreted in the urine.

3. The findings suggest that a limited enterohepatic circulation of urobilinogen occurs under physiologic conditions.

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