Urinary and Fecal Coproporphyrin Excretion in Rats. III. Excretion of Injected Coproporphyrin.* (20318)

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Previous papers have dealt with the urinary (UCP) and fecal (FCP) excretion of coproporphyrin in normal rats(1) and in rats subjected to various forms of nutritional and chemical liver injury(2). The latter group included especially rats on a choline-deficient diet and those treated with thioacetamide or with carbon tetrachloride vapor. Among these rats, only those treated with carbon tetrachloride showed even a moderate increase in coproporphyrinuria. All, however, showed considerable elevations of the ratio of urinary to fecal coproporphyrin.

Since elevation of this ratio has been reported(3,4) to correlate better with liver dysfunction than does either value alone, studies were carried out to determine the relationship between liver function and the pathway of excretion of intravenously administered coproporphyrin. The present report is concerned with an analysis of such studies in normal rats and in rats with experimentally induced liver damage. An attempt was also made to determine the pathway of excretion of injected coproporphyrin after ligation of the common bile duct and after the creation of an external biliary fistula.

Methods. 1) *Administration of Coproporphyrin.* The 48-hour UCP and FCP excretion were first determined. A solution containing 250 to 500 μg of coproporphyrin isomer I or III was then injected into a foot vein, and the UCP and FCP determinations were repeated during the following 48 hours. The coproporphyrin used was crystallized as the methyl ester, and saponified in a few ml of 7.5 N hydrochloric acid. Following neutralization of the acid with sodium acetate, the porphyrin was extracted with ethyl acetate. The latter was washed repeatedly with water and vacuum distilled to dryness. The porphyrin was dissolved either in a 0.16 M sodium carbonate solution or in a 5% sodium bicarbonate solution just before its administration in a total volume of about 1.5 ml. 2) *Production of liver damage.* The procedures used for the production of liver damage by carbon tetrachloride and by a choline-deficient diet have been described previously(2). 3) *Production of biliary obstruction and biliary fistulae.* Obstruction of the common bile duct was performed by placing double ligatures of No. 5 silk as close to the hilus as possible in order to confine the resulting marked dilatation of the bile ducts to the intrahepatic portion of the system. Creation of an external biliary fistula was accomplished at laparotomy by inserting a plastic tube (internal diameter 0.11 inch, external diameter 0.23 inch) into the common bile duct and securing it in place with silk sutures. The tubing was passed through the peritoneal cavity and by a subcutaneous route to the outside near the base of the skull. The tube was then passed through a small cork. A finger cot was fastened about the cork and formed a reservoir to collect the bile. The finger cot was held in place on the animal's back by means of a small plastic cover. Bile was aspirated from the sack, usually every 6 hours, to prevent any rise in pressure. Rats tolerate this procedure well and are free to move about in their cages. 4) *Analytical methods.* Methods employed have been described in a previous communication(1).

Results. 1) *Fate of injected coproporphyrin.* When a solution of coproporphyrin (500 μg of isomer III in 1.5 ml of 5% sodium bicarbonate) is injected intravenously into an anesthetized normal animal, the bile duct very quickly exhibits an intense red fluorescence when viewed in ultraviolet light. The intensity and rapidity of the development of fluorescence is very similar to that seen after the intravenous injection of fluorescein. In

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TABLE I. Porphyrin Excretion (μg/48 Hr) in Rats with Altered Biliary Outflow.

No. of rats	Analyses		Values Pre-op.	Values Post-op.	Procedures
2	Urine	C*	36.6	23.1	External biliary fistula (plastic tube); 500 μg coproporphyrin III intrav.
	Feces	C	41.8	18.9	
		P	252.5	66.5	
	Bile	C		259.6	
2	Urine	C	54.5	25.2	External biliary fistula (plastic tube); 500 μg coproporphyrin via stomach tube
	Feces	C	22.0	162.3	
		P	110.0	105.8	
	Bile	C		27.6	
	Intestinal content	C		119.7	
		P		30.0	
1	Urine	C	31.2	30.9	500 μg coproporphyrin III, intrav.
	Feces	C	32.0	302.4	
		P	130.0	147.2	
	Urine	C		281.4	Ligated common bile duct; 500 μg coproporphyrin III intrav.
	Feces	C		16.2	
		P		56.0	
1	Urine	C	41.6	43.4	Ligated common bile duct; (no coproporphyrin given)
	Feces	C	55.0	13.6	
		P	220.0	50.0	

C = Copro; P = Proto.

one such experiment, the bile was collected via a plastic tube inserted in the duct; the animal was kept in a state of anesthesia with ether. In 2 hours, 177 μg of coproporphyrin were recovered in the 2.1 ml of bile obtained. The concentration of coproporphyrin in the blood at the end of the 2-hour period was 3.8 μg per ml.

The changes in the urinary and fecal excretion of coproporphyrin and protoporphyrin in six male rats with various alterations of the bile duct system, are shown in Table I. These rats each weighed approximately 250 g. It is to be noted that when the bile was prevented from reaching the intestine, the coproporphyrin of the feces decreased. Since the collections were made in the immediate 48-hour postoperative period, the presence of some coproporphyrin is to be expected, because the first portion of the feces excreted represents material present in the intestinal tract at the time of surgery. When coproporphyrin is injected intravenously, the preferential route of excretion appears to be by way of the bile as judged by the recovery of the porphyrin in the bile in the case of an external biliary fistula or in the feces where the bile duct is patent. When the bile outflow is obstructed, the pigment appears in the urine. The absorption of the material from the gastrointestinal tract seems to be minimal since oral administration did not result in an increase of porphyrin in either the urine or bile. (Table I.) No more than trace amounts of protoporphyrin were present in the bile and urine samples.

2) *Effect of acute carbon tetrachloride exposure.* The excretory patterns of intravenously administered coproporphyrin I in 6 normal male and 6 normal female rats are presented in Table II. After the control values for the UCP and FCP were obtained, each rat received 500 μg of coproporphyrin I intravenously. This resulted in no appreciable increase in the UCP, while the FCP increased to a considerable extent. Approximately one-half of the injected coproporphyrin could be accounted for by the increase in the FCP.

After the above determinations were made, the rats were exposed for one hour to carbon tetrachloride vapor. Twenty-four hours later, when liver damage was at its height, coproporphyrin I was again administered. As shown in Table II the rat now exhibited an increase in the UCP equal to that of the FCP.

A similar study, employing coproporphyrin III, was conducted in another group of rats. The results are shown in Table III. Prior to

TABLE II. Excretion of Injected Coproporphyrin I by Rats before and after Production of Liver Damage by Carbon Tetrachloride.

No. of rats studied	Analysis*		Control values	Values after 500 μg coproporphyrin I, I.V. Before liver damage	Values after 500 μg coproporphyrin I, I.V. After liver damage
6 ♂	UCP	Range	30–53	21– 54	68–372
		Avg	46	41	152
	FCP	Range	23–40	144–406	39–224
		Avg	32	306	152
6 ♀	UCP	Range	12–30	19– 40	38–273
		Avg	20	30	164
	FCP	Range	19–64	204–379	110–236
		Avg	43	310	164

* Urinary (UCP) and fecal (FCP) coproporphyrin values in μg/48 hr.

TABLE III. Excretion of Injected Coproporphyrin III by Rats before and after Production of Liver Damage by Carbon Tetrachloride.

No. of rats studied	Analysis*		Control values	Values after 500 μg coproporphyrin I, I.V. Before liver damage	Values after 500 μg coproporphyrin I, I.V. After liver damage
5 ♂	UCP	Range	31–54	27– 58	51– 87
		Avg	45	46	72
	FCP	Range	16–64	318–480	133–252
		Avg	29	379	175
6 ♀	UCP	Range	11–22	15– 18	42–184
		Avg	16	17	122
	FCP	Range	5–42	215–530	21–208
		Avg	26	292	73

* Urinary (UCP) and fecal (FCP) coproporphyrin values in μg/48 hr.

the production of liver damage, the intravenous injection of 500 μg of coproporphyrin III again resulted in an increase of only the FCP. Twenty-four hours after carbon tetrachloride exposure, when liver damage was maximal, the study was repeated. The UCP values then increased slightly in the male but to a considerable extent in most of the females. As in the case of the isomer I study, less than half of the injected material subsequently appeared in the urine or feces.

3) *Effect of chronic choline deficiency.* The fate of injected coproporphyrin (isomer type III) was also studied in 6 female rats in which fatty liver and early cirrhosis existed. This form of liver damage was produced by maintaining the rats for 117 days on a choline-deficient diet. Because of the animals' failure to grow appreciably on such a diet, their average body weight was only 90 g at the time of the experiment. Therefore, only 250 μg of the porphyrin was administered. As had been noted in previous studies(2), the control UCP and FCP values were low in these animals despite the presence of severe liver damage (Table IV). No appreciable increase in either the UCP or the FCP was observed after the injection of coproporphyrin despite the fact that two successive 48-hour collections were made.

4) *Effect of post-hemorrhagic anemia.* Because rats on a choline-deficient diet exhibit anemia, it was felt that the disappearance of essentially all the injected coproporphyrin in the above-treated rats might in some way be associated with the anemia. Four rats were therefore bled after control data for the UCP and FCP were obtained. A coproporphyrin excretion study after injection of 500 μg of isomer type III was made at a time when the hemoglobin and erythrocyte counts were reduced and a mild reticulocytosis existed. As shown in Table V, results were similar to those observed in normal rats (Tables II and III); nearly half of the injected coproporphyrin was excreted in the feces.

Discussion. It would appear from these studies that about half of the intravenously administered coproporphyrin is excreted in the feces in normal rats, while essentially none is excreted in the urine. Since studies have shown a rapid increase of porphyrin in the bile of rats treated in this way, it seems certain that the injected porphyrin is excreted by the liver into the intestinal tract. The data pre-

TABLE IV. Excretion of Injected Coproporphyrin III by Rats with Fatty Livers and Early Cirrhosis (Choline Deficient Diet, 117 Days).

No. of rats studied	Analysis*		Control values	Values after 250 μg CP III, I.V. 1st 48 hr	Values after 250 μg CP III, I.V. 2nd 48 hr
6 ♀	UCP	Range	9–28	7–29	8–34
		Avg	16	19	20
	FCP	Range	1–10	10–47	7–53
		Avg	6	22	39

* Urinary (UCP) and fecal (FCP) coproporphyrin values in μg/48 hr.

TABLE V. Excretion of Injected Coproporphyrin III by Rats with Acute Post-Hemorrhagic Anemia.

Rat No.	Sex	Wt, g	Analysis*	Control values before bleeding	Values after 500 μg CP III, I.V., after bleeding	Hematological data 24 hr after bleeding (3 ml blood loss) Hb., g/100 ml	RBC	Retic., %
D 33	♀	130	UCP	44	26	8.6	4.2 × 10^6	3.0
			FCP	25	230			
34	♀	120	UCP	42	20	9.4	3.86 × "	3.8
			FCP	45	240			
37	♂	130	UCP	61	88	10.2	4.18 × "	3.4
			FCP	47	296			
38	♂	130	UCP	70	77	10.4	3.62 × "	2.4
			FCP	34	180			

* Urinary (UCP) and fecal (FCP) coproporphyrin values in μg/48 hr.

sented suggest that the ability of the functionally damaged liver to excrete coproporphyrin is impaired. This leads to reduced fecal values and to increased urinary excretion of the porphyrin.

The above findings and interpretation are consistent with the previous report of increased ratios of UCP/FCP excretion in rats with various forms of experimental liver damage(2), as well as with similar reports in humans with liver disease(3,4). However, they leave several pertinent questions unanswered: 1) What is the fate of that portion of the injected porphyrin (approximately 50 per cent) not accounted for in the excreta? 2) Why was a still larger fraction unaccounted for in the choline-deficient rats? 3) How can the presence of marked coproporphyrinuria with normal fecal coproporphyrin values in lead poisoning(2) be harmonized with the concept of diversion by altered liver function? 4) Why do normal rabbits that are injected intravenously with 500 to 1000 μg of coproporphyrin show no subsequent increase in either urinary or fecal coproporphyrin values(5)?

These questions might perhaps be answered if we knew something of the further metabolism of coproporphyrin in the body, *i.e.*, its possible position as a precursor in the synthesis of heme compounds. This possibility is being investigated by the administration of C^{14}-labelled porphyrins. Another possibility which must be considered is that the coproporphyrin occurring naturally in blood and tissues differs significantly from the injected material. Previous studies(6,1) have shown that approximately half of the freshly-passed urinary coproporphyrin is in the form of a non-fluorescing precursor which is converted to coproporphyrin by iodine, light, etc. It seems highly probable that the coproporphyrin in the body is also present in the form of some such precursor. Furthermore, since porphyrins combine readily with proteins, it may be that the porphyrin-protein complex of the native porphyrin is different from that of the injected porphyrin. The excretion pattern of the two might therefore be different.

Studies of experimental porphyria in rabbits(7-9) as well as studies in humans(10,11) have indicated two major sources of the excreted porphyrin, namely the developing red cells of the bone marrow and the liver. These studies, as well as those of patients exhibiting increased blood regeneration, suggest that the source of the porphyrin has no primary relationship to the excretory pattern observed. Thus, for example, the bone marrow is primarily involved in lead poisoning and erythropoietic (congenital) porphyria. Nevertheless, in the former condition coproporphyrin is excreted almost entirely in the urine, while in the latter, it appears chiefly in the feces.

The absence of increased urinary porphyrin excretion following the "oral" administration of coproporphyrin is in agreement with similar observations by Larson and Watson in human subjects(12). In view of the fecal recovery of only half of the administered material, however, the intestinal absorption of coproporphyrin is still possible. The use of C^{14}-labelled porphyrin in such an experiment should

yield decisive information in this regard.

Summary and conclusions. 1. Studies have been made of the urinary and fecal excretion of coproporphyrins I and III administered intravenously to rats. In the normal animal, no increase in urinary excretion occurred; approximately one-half of the injected material was excreted in the feces. 2. Coproporphyrin administered intravenously to rats was rapidly excreted in the bile via an external biliary fistula, or in the urine when complete biliary obstruction existed. Orally administered coproporphyrin did not appear to be absorbed, and approximately half was recovered from the feces. Regardless of the route of administration, only half of the coproporphyrin could be recovered. 3. In acute liver injury due to carbon tetrachloride, intravenous administration of coproporphyrin resulted in an increased urinary porphyrin excretion, with a relative reduction in the amount excreted in the feces. 4. In rats with chronic liver injury due to a choline-deficient diet virtually none of the injected porphyrin could be recovered in either urine or feces. The reason for this is unknown. 5. Rats made anemic by acute bleeding exhibited normal (*i.e.*, increased) fecal excretion of coproporphyrin III, following its intravenous administration.

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Proteolytic, Hypotensive and Thromboplastic Properties of Hypochlorite Treated Trypsin.* (20319)

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It is well known that the parenteral administration of trypsin induces a reaction similar to that of an antigen-antibody response with a sharp fall in blood pressure resulting from intravenous administration(1). That the inhibition of the proteolytic activity of the enzyme does not necessarily diminish its hypotensive effect was pointed out earlier(2). It was also reported that iodination(3,4) and treatment with formaldehyde(2) serve to decrease the hypotensive activity of trypsin more than its proteolytic or thromboplastic effects. Since the proteolytic and hypotensive properties are separable more advantageous means of accomplishing this separation were

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