the urinary and fecal coproporphyrin was type III isomer. 2. The administration of androgen to female castrate rats increased the excretion of coproporphyrin in the urine. The administration of estrogen to male castrate rats caused a decrease in the amount of coproporphyrin in the urine. 3. A diuresis, induced by substituting saline for drinking water, did not increase the urinary excretion of coproporphyrin. 4. Trace amounts of uro-type porphyrins are excreted normally in rat urine and feces. Protoporphyrin is excreted only in the feces.

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Urinary and Fecal Coproporphryn Excretion in Rats. II. Results in Experimental Liver Damage.* (20317)

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In man an increased excretion of urinary coproporphyrin is commonly associated with liver functional impairment(1,2). A study of this phenomenon in the rat was undertaken because of the extensive use of this animal in experimental investigations of liver injury. The study was designed to determine the correlation between the degree of coproporphyrinuria and the severity of hepatic damage. In a previous study of experimental nutritional liver injury (dietary necrosis), the failure to observe increased urinary coproporphyrin was ascribed tentatively to the acuity of onset of the fatal hepatic lesion(3). Therefore, in the present study other types of acute and chronic but non-fatal forms of liver injury were investigated. These included hepatic damage induced by choline deficiency, and chemical injury induced by carbon tetrachloride vapor or by ingestion of thioacetamide. The effect of lead and of Rose Bengal plus light, known to increase urinary coproporphyrin excretion in some species, was also studied in a small group of normal rats. The fecal excretion of coproporphyrin was determined in all rats to see if an increase in the urinary/fecal ratio of this porphyrin might be a more sensitive index of liver injury than the urinary value alone.

Methods. The methods for urinary and fecal collections as well as the procedures employed for the estimation of porphyrins have been described in a previous report(4). All animals were housed in individual cages throughout the course of the experiments. All

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TABLE I. Urinary and Fecal Coproporphyrin Excretion in Rats Maintained on a Choline Deficient Diet. 4 groups.

Rats studied	Analysis	Day of experiment: 4	19	26–32	41	54–70	82–84	113	134–171	195	205	219
3 ♂	UCP			9	12	14	16	23	26			
	FCP			6	4	3	6	3	2			
	Body wt, g			50	54	58	64	70	78			
2 ♀	UCP			11	30	20	21					
	FCP			6	3	6						
	Body wt, g			55	60	61	60					
2 ♂	UCP	15	36	22		26	20		11	38	13	19
	FCP	10	8	8		8	7		7	14	8	9
	Body wt, g	157	153	152		172	177		163	147	142	133
4 ♀	UCP	6	16	13		12	20		17*	30*	21†	17†
	FCP	16	8	11		6	10		10*	3*	5†	5†
	Body wt, g	128	125	124		140	143		123*	110*	106†	98†
Avg ratio UCP/FCP		1.7			4.0		4.6					

Urinary (UCP) and fecal (FCP) coproporphyrin values in μg/48 hr. Avg values listed, although separate analyses were performed on the excreta of each rat.

* Observations on 3 rats. † Observations on 2 rats.

Survival periods: Group 1—170, 184 and 198 days; Group 2—103 and 137 days; Group 3—222 and 246 days; Group 4—164, 198, 222 and 241 days.

animals received weighed amounts of the special diets employed; water was allowed *ad libitum*.

Diets employed. 1) The choline-deficient diet described by Gyorgy and Goldblatt as LVI(5) was employed. This diet contains 8% casein. Each rat received a 10 g allowance daily, with a daily supplement of 1 ml of a vit. B solution containing 20 μg of thiamin chloride, 100 μg of calcium pantothenate, 20 μg of pyridoxine, and 25 μg of riboflavin. 2) The normal diet consisted of Purina Fox-Chow. This was fed *ad libitum*. 3) The thioacetamide was added to finely-ground commercial food (Purina Fox-Chow) at a level of 0.05%. Each animal received 10 g of the diet mixture per day. *Carbon tetrachloride exposure.* Individual rats were placed in a large laboratory desiccator through which air containing approximately 50 p.p.m. of CCl_4 vapor was circulated at a rate of 3 liters per minute. In normal rats, a one-hour exposure produced a state of intoxication and, generally, a temporary hind quarter paralysis.

Results. 1. *Choline-deficient diet.* Rats maintained on choline-deficient diets develop an intense fatty infiltration of the liver. If this nutritional deficiency is long continued, a diffuse fibrosis and cirrhosis develops, as has been shown by many investigators.

Table I presents data for a group of 11 rats maintained on such a diet. The animals succumbed to the effects of the dietary deficiency at various intervals from 103 to 241 days. At autopsy all showed extensive fatty infiltration of the liver and varying degrees of fibrosis (cirrhosis). As noted in Table I, growth was markedly retarded. The weights of the 48-hour fecal samples and the volumes of the urine samples for this period were below those of well-fed normal rats. They were adequate, however, for the coproporphyrin analyses. It will be noted that the values of the FCP were consistently low throughout the entire experiment. The UCP values in the male rats were also low. Although there was a tendency toward a slight increase as the experiment progressed, the levels did not reach those shown by normal healthy rats. The females, especially those that survived longer, showed but slightly increased UCP values. In 90 control rats(4), an average urine/feces coproporphyrin ratio of 1.0 was found, average values being higher in male than in female animals. In the present study, determinations were not made prior to the institution of the choline-deficient diet. However, 23 analyses through the 32nd day showed an average UCP/FCP ratio of 1.7. From days 41-70 the average ratio in 16 analyses rose to 4.0, while the final 33 analyses from days 82-219 showed an average ratio of 4.6.

TABLE II. Influence of Choline Deficient Diets on Coproporphyrin Excretion in Rats Receiving Alcohol or Water as Drinking Fluid. 4 groups.

Rats studied	Fluid intake		Days after start of exp. 1	189	226	234	261	274	294–301	334–351	368–381
			–Normal diet–			Choline-deficient diet					
		Days after start of low choline diet				8	35	48	68–75	108–125	142–155
4 ♂	Water	UCP			43			21	28	57*	88‡
		FCP			66			17	14	10	10
		Body wt, g	90		335			263	208	197	207
"	10% alcohol	UCP			21			33	47	27	37‡
		FCP			33			9	14	13	12
		Body wt, g	80		264			253	219	210	180
2 ♀	Water	UCP			7			29	47	33†	38†
		FCP			41			20	16	9	11
		Body wt, g	101		237			198	182	161	161
"	10% alcohol	UCP		6		23	16		31	31	
		FCP		35		4	13		11	15	
		Body wt, g	75	193		189	175		186	170	
	Avg ratio UCP/FCP			0.5		—		2.5		4.1	

Urinary (UCP) and fecal (FCP) coproporphyrin values in μg/48 hr.
* Avg values for 3 rats. † Values for 1 rat. ‡ Avg values for 2 rats.
Survival periods after start of low choline diet: Group 1—84, 136, 193, 189 days; Group 2—108, 138, 150, 189 days; Group 3—120, 185 days; Group 4—150, 150+ days.

TABLE III. Coproporphyrin Excretion in Rats with Acute Liver Damage Due to Carbon Tetrachloride.

Rats studied	Avg wt, g	Analysis	Control values μg/48 hr	Values after exposure to carbon tetrachloride vapor, μg/48 hr 0-48 hr period	96-144 hr period
2 ♂	52	UCP	33	45	24
		FCP	51	19	31
2 ♀	103	UCP	14	20	11
		FCP	47	12	24
3 ♂	250	UCP	48	83	
		FCP	33	19	
3 ♀	187	UCP	13	24	15*
		FCP	34	—	26*
Avg ratio UCP/FCP			.7	3.0	.6

* Avg values for 2 rats only.

2. *Choline-deficient diet plus alcohol.* In an attempt to study the influence of alcohol on the UCP and FCP excretion, a group of 6 normal rats was given 10% alcohol in place of water as a drinking fluid. They appeared to tolerate this well and gained in weight while on an unrestricted normal diet (Table II). After 189 days and 226 days, UCP and FCP determinations were made. The values of the alcohol-treated male rats were somewhat lower than in those receiving water at this time. On the 226th day, all the animals were started on a low-choline diet. All animals lost weight. The FCP values were reduced just as in the first series of animals on this deficient diet, as above described. The UCP values rose somewhat as the experiment progressed, but again the levels were not striking and only occasionally exceeded those encountered in normal animals. The combined effect of alcohol and a deficient diet did not increase the UCP. Alcohol did not increase the degree of fibrosis or the severity of the cirrhosis in these animals as judged by

TABLE IV.
Coproporphyrin Excretion in Rats Maintained on a Normal Diet Plus Thioacetamide.

Rats studied	Analysis	Control values	Values after exp. diet (.05% thioacetamide) 35th day	77th day	Survival period, days
3 ♂	UCP	56	53	58*	42, 74, 98
	FCP	32	13	10*	
	Body wt, g	282	229	206	
2 ♀	UCP	14	33	36*	46, 77
	FCP	28	13	11*	
	Body wt, g	197	165	145	
3 ♀	UCP	14	28	37*	35, 85
	FCP	25	6	20*	
	Body wt, g	86	81	84	
1 ♂	UCP	62	36	37	78
	FCP	26	10	16	
	Body wt, g	115	118	133	
Avg ratio UCP/FCP		1.2	4.2	3.3	

Urinary (UCP) and fecal (FCP) Coproporphyrin values in μg/48 hr.
* Values for single rat.

the gross and microscopic appearance of the livers. Again the ratio of urinary to fecal coproporphyrin rose significantly on the choline-deficient diet from an average control level of 0.5 to 2.5 from days 274-301 and to 4.1 from days 334-381.

3. *Carbon tetrachloride exposure.* Ten rats were exposed to carbon tetrachloride vapor for one hour. The UCP and FCP excretion were determined before and at 0-48 and 96-144 hours after the exposure. The data shown in Table III summarize the average values for both small and large male and female rats. The period of exposure was sufficient to produce a severe but usually non-fatal form of acute liver damage. In previous studies, similar groups of rats have been sacrificed at intervals after this one-hour exposure. At 24 hours, such animals show a central form of cellular necrosis; approximately 50% of the cells of a given lobule appear necrotic. At 72 hours, recovery is well underway. If no further exposure is given, complete restitution occurs in 7 to 10 days and little evidence of the previous necrosis remains. The results of the present experiment indicate a moderate increase in the UCP values and a decrease in the FCP values during the first 48 hours, with a return toward normal in the third 48-hour period. The UCP/FCP ratio was considerably increased during the first 48-hour period. Unfortunately, laparotomies performed on the 3 adult male rats to permit liver biopsy, proved fatal. Thus the opportunity to collect urine and feces in this group during the 96-144 hour period was lost.

4. *Thioacetamide.* The effect of 0.05% thioacetamide in the diet was studied in 9 rats. This agent produces a severe form of chronic liver injury characterized by cellular necrosis, nodular regeneration, and cirrhosis(6-8). The rats succumbed at various intervals of from 35 to 98 days. Determinations of the UCP and FCP were made on the 35th and 77th days. The data are shown in Table IV. Despite the presence of severe liver injury, the only increases noted in the UCP values occurred in the females, and these were no greater than sometimes found in healthy normal female rats. Largely because of the drop in FCP values, the ratio of UCP/FCP showed a 3- to 4-fold increase. The failure of rats with various forms of acute and chronic liver damage to exhibit significant increases in coproporphyrin excretion prompted a study of the effects of other toxic agents in this species.

5. *Rose Bengal plus light.* Pimenta de Mello reported that Rose Bengal plus light exposure in rabbits resulted in a rapid and marked increase in the urinary excretion of coproporphyrin(9). This experiment was therefore repeated in rats. The exposure of a shaved area of the rat's back to unfiltered light from

TABLE V. Coproporphyrin Excretion in Rats Injected with Rose Bengal and Exposed to Ultraviolet Light.

Rat No.	Sex	Wt, g	Analysis	Control period	0-48 hr period after treatment*	Observations at 48 hr: Hematocrit, %	Observations at 48 hr: Biopsy of liver
B 37	♂	270	UCP	14	23	40.6	Normal
			FCP	32	31		
39	♂	250	UCP	24	12	43	"
			FCP	33	13		
40	♀	190	UCP	12	19	43.7	"
			FCP	14	19		
41	♀	145	UCP	16	20	40	"
			FCP	14	35		
42	♀	196	UCP	10	19	—	"
			FCP	17	19		
	Avg ratio UCP/FCP			.75	.85		

Urinary (UCP) and fecal (FCP) coproporphyrin values in μg/48 hr.
* Exposure to unfiltered light from AH-4 mercury lamp: 10 min. at distance 12 in. to 2" × 2" shaved area on back on animal.

TABLE VI. Coproporphyrin Excretion in Rats Following Lead Intoxication.

No. of rats	Sex	Route of administration	Analysis	Control values, μg/48 hr	Values after lead inj., μg/48 hr: 4th to 6th day	Values after lead inj., μg/48 hr: 8th to 10th day
2	♂	Intraper.*	UCP	17		207
			FCP	26		25
2	♀	" *	UCP	16		66
			FCP	18		30
6	♂	Intrav.†	UCP	53	150	
			FCP	43	18	
6	♀	" †	UCP	23	70	
			FCP	40	14	
	Avg ratio UCP/FCP			0.86	6.4	

* 10 mg lead acetate/100 g body wt.
† 2 " " " " " ".

an AH-4 mercury lamp immediately followed the intravenous injection of Rose Bengal in a dose of 10 mg per 100 g body weight. Results obtained in 5 animals are summarized in Table V. As noted, no appreciable increase was found in the excretion of UCP or FCP, or in their ratio. It is noteworthy, too, that unlike Pimenta de Mello's rabbits, these rats showed no evidence of a significant hemolytic reaction. No pathological changes were noted in their livers at the end of a 48-hour period.

6. *Lead.* Lead acetate was administered to rats both by intravenous and intraperitoneal routes. The data obtained are presented in Table VI. The intraperitoneal administration produced a severe chemical peritonitis. The liver was covered by a heavy exudate; despite this, the parenchymal cells appeared normal on histologic examination. The intravenous injection of 0.4 or 0.5 ml of a 1% solution of the lead salt was well tolerated by 6 male and 6 female rats. In 2 additional animals similarly treated an hepatic lobectomy and a unilateral nephrectomy was performed at 24 hours and the animals were sacrificed at the end of 72 hours. A study of the liver sections revealed a minimal degree of fatty infiltration at 24 hours; this had disappeared at 72 hours. The kidneys appeared normal on microscopic examination. As noted in Table VI, all animals responded by a marked increase in the UCP. The values for the FCP

remained within the normal range.

Discussion. Despite the production of histologically demonstrable hepatic lesions, it is evident that choline deficiency, carbon tetrachloride, and thioacetamide produced little if any increase in the urinary excretion of coproporphyrin in the rats studied. This is quite unlike results observed in humans with various types of liver disease(1,2). These rats did, however, show significant increases in the average ratios of urinary to fecal coproporphyrin excretion, though a considerable amount of individual variation in values was observed. This observation tends to confirm the suggestion(10,11) that elevation of this ratio may be of greater significance in liver disease than is either the urinary coproporphyrin or fecal coproporphyrin value alone. Further confirmation of this is offered in paper III of this series(12).

As regards the effect of lead poisoning, at least, it seems that the porphyrin excretion of the rat is similar to that of other species studied. On the other hand, the response of the rat to Rose Bengal and light is quite unlike that reported for the rabbit.

No attempt has been made to investigate the effect of hepatotoxic agents on the excretion of uroporphyrin in rats since the studies reported here were completed before the demonstration that uro-type porphyrins are normally found in rat excreta(4).

Summary and conclusions. 1. Despite the presence of severe liver involvement, no consistently significant increases were noted in the excretion of urinary or fecal coproporphyrin in rats fed a choline-deficient diet or thioacetamide. Average 3- to 4-fold increases, however, were observed in the ratio of urinary to fecal coproporphyrin. 2. Essentially similar results were obtained in choline-deficient rats maintained on either alcohol or water as drinking fluid. 3. Transitory increases were found in the urinary coproporphyrin and the urinary/fecal coproporphyrin ratio following acute exposure to carbon tetrachloride vapor. 4. The combination of Rose Bengal and ultraviolet irradiation failed to produce any increase in urinary porphyrin excretion in rats. 5. The injection of lead in rats produced a striking increase in the excretion of urinary coproporphyrin.

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