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Urinary and Fecal Coproporphyrin Excretion in Rats: I. Results in Normals and Castrates.* (20316)

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An increase in the excretion of urinary coproporphyrin has been referred to frequently as a sensitive index of liver functional impairment(1). In view of the extensive employment of the white rat in laboratory studies of liver damage, an investigation of this animal's excretion of coproporphyrin was conducted. As a preliminary step, it was necessary to determine the pattern of excretion in the normal well-fed animal. In the course of these investigations, normal male animals were observed to excrete more coproporphyrin in the urine than did females. Accordingly, the effects of castration and the influence of androgenic and estrogenic substances were studied. Since coproporphyrin is excreted both in the urine (UCP) and the feces (FCP), simultaneous measurements of each were made in all experiments.

Methods. In all the experiments, white rats of a uniform strain (Sprague-Dawley) were employed. The animals were fed a stock diet (Purina Fox-chow) *ad libitum* and had free access to water. The animals under study were housed in individual metal cages. In all experiments, 48 hour collections of urine and feces were made for the coproporphyrin analyses. During the collection periods, the rats were housed in individual cages suspended over large glass funnels (25 cm diameter). The associated all-glass unit used for separation of urine and feces was essentially similar to that described originally by Gross and Connel(2). The successful separation of these two forms of excreta was deemed of utmost importance in a study that involved the separate determination of a pigment common to both. The collections were interrupted for 60 minutes at the end of the first 24 hours to permit feeding of the animal and to store the first day's urine and fecal specimens. The sides of the funnels were rinsed with 10 ml of water at the end of each 24-hour period. The washings, the urine and the feces were immediately refrigerated. No preservatives were employed. The test tubes that received the voided urine were painted black to prevent exposure of the contents to light. The urine and the washings were stored in brown bottles while in the refrigerator. The urine and the washings for the 48-hour period were pooled for analysis. Although average values are given in most of the data reported, individual analyses of the 48-hour urine and fecal collections of each rat were made. Plastic cages with glass rod bottoms were used in the early experiments in order to avoid contact of the urine with metal. This was done to reduce the tendency to form metal coproporphyrin complexes. However,

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TABLE I. Urinary and Fecal Coproporphyrin Excretion in Normal (Sprague-Dawley) Rats.

Analysis		Stock A		Stock B	
		Males	Females	Males	Females
UCP	No.	33	31	25	16
	Range	14–67	6–30	18–70	14–52
	Mean	40 ± 16	14 ± 5	47 ± 13	30 ± 12
FCP	No.	32	31	13	16
	Range	11–91	12–102	16–52	17–45
	Mean	40 ± 22	46 ± 21	34 ± 11	33 ± 9
UCP/FCP	No.	32	30	12	16
	Range	0.3–2.9	0.1–1.0	0.9–2.2	0.4–1.7
	Mean	1.3 ± 0.7	0.4 ± 0.2	1.7 ± 0.4	1.0 ± 0.4

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

when metal cages were later employed, it was noted that the only significant metal complex present was that of zinc, and this is completely split by the extraction procedure employed. The copper complex of coproporphyrin comprised less than 5% of the total in those instances in which it was specifically sought. (Its concentration was determined by conversion of the non-fluorescing copper complex to free porphyrin by evaporation of the completely extracted ethyl acetate to dryness and solution of the residue in concentrated sulfuric acid.) The estimation of the fecal coproporphyrin was conducted according to the method of Schwartz and Zagaria(3). This method was slightly modified to permit its use in the analyses of the urine samples. During the analyses of both urine and feces, the initial 1.5 N HCl extracts were each diluted to a concentration of 0.12 N HCl and extracted with chloroform to remove protoporphyrin and deuteroporphyrin. The completeness of the separation of the coproporphyrin from these two porphyrins was checked by examining the red fluorescence of the chloroform washings under filtered near-ultraviolet light† (405 mu). The coproporphyrin was quantitated fluorimetrically(4). Coproporphyrin precursor was determined by comparing results obtained with and without an iodine wash(5). Estimation of the coproporphyrin isomer distribution was performed on the pooled final extracts of urine and fecal specimens. Procedure B of the method described by Schwartz and coworkers(6) was employed. Studies to be reported in detail elsewhere have revealed that results obtained with this procedure are dependent to an appreciable extent on the purity of the distilled water used. Appropriate controls were therefore analyzed to establish the slope of the calibration curve obtained under the conditions employed for the unknown samples. Even with these precautions, however, accuracy is reduced in mixtures containing over 75% of type III isomer. Values in the range of 75-100% type III isomer are therefore reported here as having more than 75% type III.

Results. I. Studies of normal rats. A. *Total coproporphyrin excretion:* The data for the urinary and fecal coproporphyrin excretion (UCP and FCP) in μg per 48 hours are summarized in Table I. The earlier studies employed rats obtained from a Minnesota laboratory (Stock A), while more recently the rats have been obtained from a Wisconsin laboratory (Stock B). As seen in Table I, male rats excreted significantly greater amounts of urinary coproporphyrin than did female rats from both stocks. Mean FCP values were approximately equal in the two sexes. The mean UCP/FCP ratios in the different groups ranged from 0.4 to 1.7, being significantly higher in the male rats. The reason for the differences observed between the two stocks (especially in the female UCP) is unknown. No significant correlation was found between weight and UCP excretion, though FCP excretion was reduced in young rats of both sexes. These data are summarized in Table II for rats of Stock A only, since the number and weight distribution of rats of Stock B is insufficient for a similar analysis.

†Model BL-2, Blacklight Products, Chicago.

TABLE II. Relationship of Weight to UCP and FCP Excretion in Normal Rats (Stock A).

Sex	Wt, g	UCP No.	UCP Avg μg/48 hr	FCP No.	FCP Avg μg/48 hr
♂	57–105	6	38	6	29
	195–246	13	38	13	45
	257–370	14	41	13	46
♀	74–115	5	14	7	31
	153–200	16	14	14	49
	219–250	10	13	10	51

B. *Excretion of individual coproporphyrin isomers (I and III):* Previous reports of normal rats urinary coproporphyrin have indicated that it consists predominantly of the type III isomer(7,8). In the present study a pooled 5-day collection of urine from 5 normal adult male rats contained a total of 282 μg of coproporphyrin. Isomer analyses of this porphyrin revealed that more than 75% was type III. This was confirmed by the isolation of the tetramethyl ester, yielding typical rosettes with a melting point of 138-142°C. The pooled feces of the same rats contained 186 μg of coproporphyrin, over 75% of which was shown to be the type III isomer. Again typical rosettes of coproporphyrin III methyl ester were isolated. Similar results were obtained from the pooled feces of 2 adult female rats.

C. *Excretion of urinary coproporphyrin precursor:* In several dozen analyses, no significant amounts of urinary coproporphyrin precursor(5) were found in urine collected in the usual way except in 2 instances, where 6 and 20% of the total porphyrin was in precursor form at the time of analysis. However, by collecting the urine in tubes immersed in dry ice to freeze the urine instantly, coproporphyrin precursor accounted for an average of 33% of the total in 6 female rats and of 24% in 6 male rats. These values are within the range of those found in human urine collected similarly(5).

D. *Excretion of other porphyrins:* Examination of both urine and feces has repeatedly demonstrated the presence of small amounts of an ether-insoluble, uro-type porphyrin. It has not yet been crystallized for more precise identification. No protoporphyrin or other chloroform-soluble porphyrins could be demonstrated in several hundred ml of pooled urine from both male and female rats. Trace amounts may, however, be found in the presence of very slight fecal contamination.

E. *Relationship of urine volume to coproporphyrin excretion:* The relationship of urine volume to the UCP was investigated by inducing a diuresis in rats in which UCP and FCP values had been previously determined. 0.8% saline solution was substituted for drinking water for one week; urine and fecal coproporphyrin analyses were then repeated. The results are presented in Table III. Although the urine volumes increased, the values for the UCP increased but very little. The reason for the drop in FCP during saline diuresis is uncertain.

II. Studies of castrated rats. Since the normal male rat exhibited an average UCP value nearly twice that of the normal female, the effect of castration was investigated. Control values were obtained in 10 rats; the animals were then castrated. In the 39 days that followed, 3 UCP-FCP determinations were made. The average UCP values rose slightly in both groups of animals. Beginning on the 60th day, the 4 male castrated rats re-

TABLE III. Effect of Saline Diuresis on Urinary and Fecal Coproporphyrin Excretion in Normal Rats.

No. rats		Body wt, g	Urine vol, ml/48 hr	UCP, μg/48 hr	Feces wt, g/48 hr	FCP, μg/48 hr	Drinking fluid
6 ♂	Range	250–295	9–46	33–67	3–23	30–69	Water
	Avg	270	20.5	49	7.6	49	
	Range		43–150	34–64	6–18	11–49	.8% saline
	Avg		78	53	8.6	32	
6 ♀	Range	190–207	9–25	6–22	2–11	43–79	Water
	Avg		15.7	11	5.9	64	
	Range		10–78	11–16	4–9.5	29–50	.8% saline
	Avg		42	14	6.0	35	

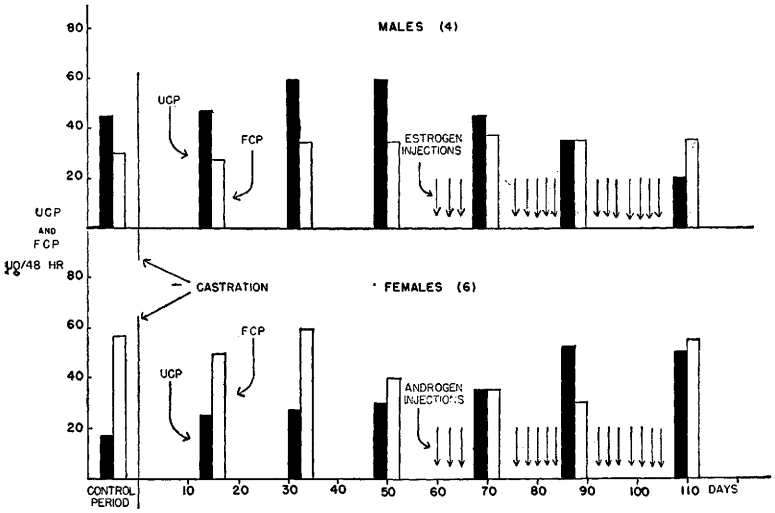

FIG. 1. Effect of castration and administration of estrogen or androgen on excretion of urinary and fecal coproporphyrin in rats.

ceived a series of intramuscular injections of 100 I.U. of an estrogen (Theelin). The 6 female castrates received intramuscular injections of an androgen (0.1 mg testosterone propionate). By the 106th day, 17 injections had been given. At that time the UCP values for the males had decreased from an average control value of 44 μg to 20 μg per 48 hours. The female rats showed an average increase of the UCP from 17 μg to 51 μg per 48 hours. The FCP values in both groups remained fairly constant. The results are shown in Fig. 1.

Discussion. The normal well-fed rat excretes a relatively large amount of coproporphyrin in the urine, average per diem UCP values being comparable to those seen in normal rabbits. The values in normal human adult males, determined by a slightly modified method, average only about 8 times as much, or approximately 160 μg per day(4,9). Though the type I isomer has been shown to be preponderant in human urine(10), the type III isomer predominates in both urine and feces of rats. In this respect, too, the rat is comparable to the rabbit(3).

The variation of the UCP values in the 2 sexes was not anticipated. So far as is known, this difference had not been reported previously, although recent observations in humans, in this laboratory, have indicated significantly lower values in females(9). The significant alteration of the UCP value by castration and estrogen or androgen injections in the present study indicates that observed sex differences are not merely chance observations in the group of animals studied. The mechanism or the site of action of estrogen or androgen in this particular respect is unknown. A primary effect upon the liver would appear to be most likely, since the excretory pattern of coproporphyrin is related to liver functions (11,12) and since the above agents are known to influence some hepatic functions under certain conditions.

Conclusions. 1. Excretion of coproporphyrin in the urine and feces of normal rats has been measured in 2 stocks of Sprague-Dawley rats. Collections periods of 48 hours were made. Male rats of these 2 stocks excreted an average of 40 ± 16 and 47 ± 13 μg of urinary coproporphyrin per 48 hours, respectively, while the female rats excreted an average of only 14 ± 5 and 30 ± 12 μg of urinary coproporphyrin per 48 hours, respectively. There was no significant correlation between body weight and urinary coproporphyrin excretion. No significant sex difference was found in the fecal excretion of coproporphyrin; mean values in the various groups ranged from 33 ± 9 to 46 ± 21 μg per 48 hours. Reduced values were found in both male and female rats weighing less than 115 g. In both males and females, over 75% of

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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