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The Sex-Related Difference in Perfluorooctanoate Excretion in the Rat[1] (41476)

H. HANHIJÄRVI,* R. H. OPHAUG,† AND L. SINGER†,[2]

Department of Dentistry, University of Kuopio, P.O.B. 138, SF-70101 Kuopio 10, Finland, and †Biochemistry Program, School of Dentistry, University of Minnesota, 515 Delaware Street Southeast, Minneapolis, Minnesota 55455

Abstract. The urinary excretion of perfluorooctanoic acid (PFO) by male and female rats was investigated. Female rats excreted 76 ± 2.7 (SEM)% of a 2-mg dose of nonionic fluorine (as PFO) in the urine in 24-hr whereas male rats excreted only 9.2 ± 3.5% of the dose. The PFO clearance, inulin clearance, net excretion rate of PFO, and the glomerular filtration rate of PFO were measured. The effect of probenecid, an inhibitor of the organic acid transport system, on these measurements was also determined. In female rats the PFO clearance was severalfold greater than the inulin clearance and the clearance of PFO was markedly reduced by probenecid. Conversely, in male rats the PFO clearance was only a fraction of the inulin clearance and was virtually unaffected by probenecid. The data indicate that female rats are able to rapidly eliminate PFO in the urine by an active secretory mechanism which is inhibited by probenecid. In male rats this secretory mechanism is either absent or relatively inactive. This difference in PFO excretion by the male and female may explain the sex-related difference in PFO toxicity in which male rats are more susceptible to high doses than females.

Guy *et al.* have reported the results of attempts to isolate and characterize the compound(s) comprising the nonionic fluorine fraction of human serum (1). They indicate that these compounds are mainly perfluoro fatty acid (C_6-C_8) derivatives and that the major fluorocarbon isolated from human serum resembled perfluorooctanoic acid ($C_7F_{15}COOH$). Since derivatives of perfluorooctanoate (PFO) are widely used commercially it is important to study its behavior in living organisms. In 1980 Ophaug and Singer reported that PFO, administered by stomach intubation to female rats, was rapidly excreted into the urine and by 96 hr only traces remained in the blood (2). Other investigators have fed diets containing 0–1000 ppm of ammonium perfluorooctanoate to rats for a period of 90 days (3). Following an overnight fast the serum of male rats contained 21–49 ppm of PFO whereas the serum of similarly treated females contained 0.15–0.65 ppm. These results confirmed the ability of female rats to rapidly dispose of rather large doses of PFO and illustrate a dramatic sex difference in the metabolic handling of this fluorocarbon by rats. Janssen *et al.* have previously shown that there is also a sex-related difference in the elimination rate of 1-aminocyclohexanecarboxylic acid (ACHC) in rats (4). The principal aim of this investigation was to determine whether there is a sex-related difference in PFO excretion by the rat kidney.

Materials and Methods. Holtzman rats fed rat chow (Purina) and tap water *ad libitum* were employed in all experiments.

Four male and six female rats were administered 2 ml of an aqueous solution containing 2 mg of nonionic fluorine as PFO by stomach intubation. Seven female rats were administered 2 ml of distilled water as controls. The animals were then placed in individual metabolic cages and fed rat chow and tap water. A few crystals of thymol were added to the urine containers to inhibit bacterial growth during the collection period. After 24 hr the animals were sacrificed by cardiac puncture. The blood was allowed to clot and the serum was collected after centrifugation. The volume of the urine collections, including the volume of

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[2] To whom correspondence should be addressed.

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water used to rinse the metabolic cages was recorded. The ionic fluoride content of the serum and urine was determined at pH 5.0 with the fluoride ion-specific electrode (5, 6). The total fluorine content of the serum and urine was determined by the oxygen-bomb reverse extraction technique (7). The nonionic fluorine level of the serum and urine was calculated as the difference between the ionic and total fluorine levels.

For clearance studies of PFO and inulin the rats were anesthetized with Inactin (80–90 mg/kg ip), a barbituric acid derivative which gives a 3- to 4-hr anesthesia. The femoral vein was cannulated for continuous infusion of 5% mannitol in isotonic saline and the femoral artery was cannulated for drawing blood samples. In order to obtain serial collections of urine the urinary bladder was also cannulated. Intravenous (iv) priming doses of 5.2–5.6 mg of [1-^{14}C] ammonium perfluorooctanoate (sp act 0.5 μCi/mg) and 8.8 μg of tritiated inulin (*methoxy*-^{3}H, sp act 114 μCi/mg) were given to each animal. The radiolabeled inulin and PFO in 5% mannitol in isotonic saline was then infused at a rate of 0.21 ml/min. An additional 0.42–0.63 mg/hr of ^{14}C-labeled PFO and 9.6 μg/hr of tritiated inulin was infused during the experiments. After a 45-min equilibration period the first plasma sample was collected for clearance calculations. Urine specimens were collected over 10-min intervals and additional arterial blood samples were obtained at the midpoint of each collection period. When the urine and serum collections for the clearance study were complete probenecid was administered (65–68 mg/kg ip) and, after 20–30 min, additional consecutive 10-min clearance tests were performed to test the effects of probenecid on the organic acid transport system.

In the cumulative excretion study the rats were prepared as described for the clearance tests except that arterial cannulation was not needed. The rats were dosed iv with a mixture of radiolabeled PFO (10–20%) and unlabeled PFO (80–90%). Five percent mannitol in isotonic saline was infused at a rate of 0.081 ml/min and urine specimens were collected over 30-min intervals. The effect of probenecid was assessed by administering 65–68 mg/kg ip at least 30 min prior to the administration of PFO.

The blood samples were centrifuged and 20 μl of plasma was removed for radioactivity determinations. The rest of the plasma sample was transferred to Amicon Centriflo 2100 CF 50 ultrafiltration cones and centrifuged. These membranes retain molecules with a molecular weight greater than 50,000 daltons. Fifty microliters of the ultrafiltrate was also prepared for radioactive determination as was 50 μl of each urine sample.

The ^{3}H (inulin) and/or ^{14}C (PFO) activity in each sample was counted in a dual-channel Packard Tri-Carb liquid scintillation spectrometer. A series of quenched standards and the [^{3}H]inulin and [^{14}C]PFO standards were also counted. The count rates obtained for all samples were corrected for background, quenching (by use of the automatic external standard method), ^{3}H spillover into the ^{14}C counting channel, and ^{14}C spillover in the ^{3}H counting channel. All samples were counted until 20,000 counts had been recorded.

The clearances (C_L) were calculated using

$$C_L \text{ (ml/min/100 g)} = \frac{U \times V}{P \times W},$$

where

U = urinary concentration of the compound (μg/ml),
V = urine flow rate (ml/min),
P = plasma concentration of the unbound compound (μg/ml),
W = animal weight (g)/100.

The net excretion rate (NE) was calculated (μg/min/100 g) using

$$NE = \frac{U \times V}{W},$$

[3] [1-^{14}C]Perfluorooctanoic acid (ammonium salt) was supplied by Minnesota Mining and Manufacturing Company, St. Paul, Minn. 55101.

where

U = urinary concentration of the compound (μg/ml),
V = urine flow rate (ml/min),
W = animal weight (g)/100.

The cumulative excretion percentage was calculated as

$$\% = \frac{U \times VF}{D} \times 100,$$

where

U = urinary concentration of the compound (μg/ml),
VF = total urine volume (ml),
D = total dose of the compound (μg).

The glomerular filtration rate (F) of PFO was obtained from

$$F\ (\mu g/min/100\ g) = P \times C_{L\ (inulin)},$$

where

P = plasma concentration of the unbound compound (μg/ml),
C_L = inulin clearance (ml/min/100 g).

All the tests were performed at least twice unless otherwise stated.

Results. The ionic and nonionic fluorine levels of the serum and the percentage of the dose of nonionic fluorine excreted in the urine 24 hr after administration of 2 mg of nonionic fluorine, as PFO, to male and female rats are presented in Table I. Twenty-four hours after administration of the dose, female rats had excreted 76 ± 2.7% of the dose in the urine and had a mean serum nonionic fluorine level of 0.35 ppm. Although this serum nonionic fluorine level is considerably higher than that observed in female rats which did not receive a dose of nonionic fluorine (0.07 ± 0.02 ppm), male rats had serum nonionic fluorine levels which were much higher (44.0 ± 1.7 ppm) and had excreted only 9.2 ± 3.5% of the dose of nonionic fluorine in the urine. The serum ionic fluoride levels of male and female rats given PFO were not different from that of undosed female rats.

PFO was bound to a similar extent in the plasma of male and female rats. A mean of 97.5 ± 0.25% (SEM, N = 16) of the PFO in the plasma was bound. The results of clearance studies on male and female rats are shown in Table II. In repeated tests it became obvious that there was a crucial difference in PFO clearance and the PFO/inulin clearance ratio between sexes. The PFO clearance in female rats was several times greater than the inulin clearance. The administration of probenecid (65–68 mg/kg ip), which strongly inhibits the renal active secretion system for organic acids (9), dramatically reduced the clearance ratio in female rats. The net excretion of PFO was reduced from 4.6 to 0.13 μg/min/100 g following the administration of probenecid. In male rats, however, the PFO/inulin clearance ratio and the net excretion of PFO were virtually unaffected by probenecid.

TABLE I. SERUM IONIC FLUORIDE AND NONIONIC FLUORINE LEVELS AND URINARY EXCRETION OF NONIONIC FLUORINE 24 HR AFTER ADMINISTRATION[a] OF A 2-MG DOSE OF NONIONIC FLUORINE AS PFO TO MALE AND FEMALE RATS

Sex	Treatment	Serum		Percentage of dose excreted in urine
		Ionic fluoride (ppm)	Nonionic fluorine (ppm)	
Female	No dose	0.032 ± 0.003[b] (7)	0.07 ± 0.02 (5)	—
Female	Dosed	0.020 ± 0.003 (6)	0.35 ± 0.11 (6)	76 ± 2.7 (4)
Male	Dosed	0.033 ± 0.003 (4)	44.0 ± 1.7 (4)	9.2 ± 3.5 (4)

[a] Gastric intubation
[b] Mean ± SEM (No. of animals).

TABLE II. EFFECT OF PROBENECID (65–68 MG/KG IP) ON THE PFO/INULIN CLEARANCE RATIO, NET PFO EXCRETION, AND FILTERED PFO IN MALE AND FEMALE RATS

	Female		Male	
	No probenecid	After probenecid	No probenecid	After probenecid
PFO clearance (ml/min/100 g)	5.8[a] (5.5[b]–6.0)	0.11 (0.08–0.14)	0.17 (0.16–0.17)	0.10 (0.09–0.11)
PFO clearance inulin clearance	14.5 (8.2–20.7)	0.46 (0.43–0.48)	0.22 (0.17–0.26)	0.12 (0.11–0.13)
Net PFO excretion (μg/min/mg)	4.6 (3.9–5.2)	0.13 (0.09–0.17)	0.17 (0.13–0.21)	0.12 (0.11–0.12)
Filtered fraction (μg/min/100 g)	0.42 (0.20–0.64)	0.29 (0.21–0.37)	0.75 (0.72–0.77)	1.00 (0.91–1.10)

[a] Mean (range of values) for two animals.
[b] Mean values for each animal of two to four determinations.

Table III presents the data obtained in cumulative excretion studies of PFO in the urine of male and female rats over a 7-hr period. Female rats were observed to excrete 76% of the administered PFO (23–25 mg/kg) whereas male rats excreted only 7.8% of the dose. Intraperitoneal injection of probenecid (65–68 mg/kg) given at least 30 min before administration of the PFO modified the cumulative excretion curve for males only slightly. In female rats, however, probenecid markedly reduced PFO elimination to 11.8%.

Discussion. Griffith and Long have clearly shown a sex-related difference in PFO toxicity in rats being fed PFO in their diet (3). The liver appeared to be the target organ in rats, and males were found to be more susceptible to high doses of PFO than females. The PFO concentration in pooled plasma and liver specimens was considerably higher in male rats than in similarly treated females.

Twenty-four hours after receiving a 2-mg dose of nonionic fluorine (as PFO) male rats had serum nonionic fluorine levels that were more than 100-fold higher than that of similarly treated females (Table I). The serum ionic fluoride levels of male and female rats were not significantly increased following administration of the PFO. This provides good evidence for the metabolic stability of PFO in rats. The conclusion that PFO is metabolically stable in rats is supported by the demonstration of quantitative recovery of nonionic fluorine in the urine and feces of female rats given PFO (2). In addition, Hagen *et al.* have recently demonstrated the accumulation of PFO in the serum of male rats given a single oral dose of 1H,1H,2H,2H-perfluorodecanol (8). In contrast to female rats which excreted 76 ±

TABLE III. EFFECT OF PROBENECID (65–68 MG/KG IP) ON THE CUMULATIVE URINARY EXCRETION OF PFO IN MALE AND FEMALE RATS[a]

	Female		Male	
Hours	No probenecid	After probenecid	No probenecid	After probenecid
1	21.6 (20.2–22.9)[a]	1.1 (1.1–1.1)	1.4 (0.9–1.8)	1.0 (0.9–1.1)
2	36.3 (32.7–39.9)	2.5 (2.2–2.7)	2.4 (1.6–3.1)	1.7 (1.5–1.8)
3	46.1 (38.6–53.5)	3.6 (3.1–4.1)	3.4 (2.4–4.3)	2.4 (2.2–2.6)
4	56.5 (49.5–63.4)	5.3 (4.0–6.6)	4.5 (3.5–5.4)	3.1 (2.8–3.3)
5	65.7 (59.3–72.0)	7.2 (4.8–9.6)	5.7 (4.9–6.4)	3.7 (3.3–4.0)
6	71.9 (64.8–78.9)	9.2 (5.8–12.6)	6.8 (6.3–7.3)	4.4 (3.9–4.8)
7	76.2 (68.9–83.5)	11.8 (6.6–17.0)	7.8 (7.3–8.2)	5.0 (4.4–5.5)

[a] Results are percentage of dose excreted.
[b] Mean (range of values) for two animals.

2.7% of the PFO dose in the urine after 24 hr, male rats excreted only 9.2 ± 3.5% of the dose (Table I). These data indicate that the sex-related difference in PFO toxicity in rats (3) is due to the relatively slow urinary excretion of PFO in male rats.

Since inulin is excreted only by glomerular filtration and not actively secreted in renal tubuli, the observation of PFO/inulin clearance ratios that were substantially greater than 1.0 for female rats (Table II) provides evidence that, in the female rat, PFO is excreted in part by an active secretion mechanism. The fact that probenecid rapidly decreases the PFO/inulin clearance ratio from 14.5 to 0.46 strongly supports this conclusion. Since the PFO/inulin clearance ratio for male rats was less than 1.0 and not significantly altered by the administration of probenecid it appears that active tubular secretion of PFO in males either does not occur or occurs at an insignificant rate. Additionally, both the female rat after receiving probenecid and the male rat throughout the clearance studies had lower PFO than inulin clearance. This indicates that there is partial tubular reabsorption of PFO in both sexes. Janssen *et al.* have shown that there is a sex-related difference in the tubular reabsorption of 1-aminocyclohexanecarboxylic acid (ACHC) in the rat kidney (4). ACHC is not bound to plasma proteins whereas in the present study 97.5 ± 0.25% (SEM) of the PFO was bound. Thus the excretion of ACHC by glomerular filtration occurs to a much greater extent than PFO. Our data indicate that there is a sex-related difference in the active secretion of PFO. It is therefore possible that female rats also actively secrete ACHC to a greater extent than male rats.

The cumulative urinary excretion data in Table III illustrates the striking sex-related difference in PFO elimination. At doses of 23–25 mg/kg male rats, because of a limited or completely inactive secretion mechanism, are able to net-excrete in 7 hr only about 10% of the amount of PFO excreted by females. Although probenecid had little effect on the cumulation excretion curve for males a low level of active tubular secretion of PFO cannot be ruled out. It is possible that probenecid, as an acid, slightly increases the tubular reabsorption of PFO and partially masks the active secretion.

Ophaug and Singer found that female rats excreted 61% of the administered dose of nonionic fluorine (PFO) in 8 hr (2). The more rapid excretion (76% in 7 hr) observed for female rats in the cumulative excretion studies (Table III) is probably due to the fact that the animals in the present study were infused with 5% mannitol in isotonic saline during the excretion period and that the PFO was administered iv rather than by gastric intubation. After the administration of probenecid to female rats the rate of excretion of PFO decreased dramatically and after 1 hr there was no difference in PFO elimination between sexes. Griffith and Long observed that rhesus monkeys do not exhibit a sex-related difference in the elimination of PFO (3). Serum PFO levels (following an overnight fast) ranged from 45 to 71 ppm for males receiving 3 or 10 mg PFO/kg/day as compared to levels of 50 to 79 ppm for corresponding females. The high serum levels of PFO found in both sexes of rhesus monkey closely reflect the situation found in male rats in that they appear to eliminate a dose of PFO rather slowly. One might speculate, therefore that the probenecid-sensitive active secretory system we have observed in female rats is absent or inactive in both sexes of the rhesus monkey.

Based upon these data it is concluded that the female rat possesses an active secretory mechanism which rapidly eliminated PFO from the body. This secretory mechanism is lacking or relatively inactive in male rats and accounts for the greater toxicity of PFO in male rats.

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