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Metabolic Handling of Perfluorooctanoic Acid in Rats (40715)

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It now generally accepted that fluorine in human serum exists in both ionic and nonionic forms. This conclusion was derived from the observation of Taves that the total fluorine content of serum determined with the fluoride-ion electrode after open ashing was greater than the values obtained with procedures which measure ionic fluoride and do not involve ashing of the specimen (1). The nonionic fraction of serum fluorine was found by Taves to be nonexchangeable with radioactive fluoride ($^{18}F^-$), and not ultrafilterable from human serum (1, 2). Electrophoresis of human plasma at pH 9.0 resulted in a clear separation of inorganic fluoride from the nonionic fluorine which migrated with albumin (3). In human serum, the nonionic fluorine normally constitutes at least 50% of the total fluorine (4–7). Under conditions where the aqueous fluoride intake is high the ionic form may predominate (7).

Guy *et al.* have recently isolated and characterized the compounds which comprise the major portion of the nonionic fluorine fraction of human serum and found them to be predominantly perfluoro-fatty acid derivatives containing six to eight carbons (7). They indicated that human serum also contains much smaller quantities of other uncharacterized organic fluorocarbons. There is little known as to metabolic fate of perfluorinated fatty acids. The present study with the rat was undertaken to investigate the metabolic handling of one such perfluorinated fatty acid, perfluorooctanoic acid, an 8-carbon fatty acid containing 15 fluorine atoms.

Materials and methods. Female Holtzman rats, weighing approximately 250 g, were administered 2 ml of an aqueous solution containing 2 mg of nonionic fluorine as perfluoroctanoic acid [1] by stomach intubation. The animals were then placed in individual metabolic cages and fed rat chow (Purina) and tap water (1 ppm fluoride) *ad libitum* for 4.5, 8, 24, or 52.5 hr. In addition, four rats were placed in metabolic cages and fed a low fluoride (<0.5 ppm) diet and distilled water for a period of 96 hr. This resulted in a substantial decrease in the ionic fluoride content of the feces and facilitated the analysis for nonionic fluorine. A few crystals of thymol were added to the urine containers to inhibit bacterial growth during the collection period. At the end of the experimental period the urine and feces were collected and the anesthetized animals were sacrificed by cardiac puncture. The blood was allowed to clot and the serum was collected after centrifugation and stored frozen until analyzed. Urine, serum, and feces were also obtained from undosed animals to provide baseline data.

The volumes of urine collections which included water needed to rinse the metabolic cages and the weight of the feces were recorded. A small known quantity of distilled water was added to the feces samples which were then homogenized to a thick slurry.

Fluoride determinations. The serum and urine were analyzed for ionic fluoride at pH 5.0 with the fluoride ion-specific electrode (8, 9). The ionic fluoride in the diffusate of the feces was determined at pH 5.0 with the fluoride electrode after isolation of the fluoride by diffusion from perchloric acid at 60°C (10). The fluoride in perfluorooctanoic acid is not acid labile under these conditions. [2] The total fluorine content of serum, urine and feces was determined with the

[1] Perfluorooctanoic acid (a mixture of linear and branched isomers) was kindly supplied by Minnesota Mining and Manufacturing Company, St. Paul, Minn. 55101.

[2] R. H. Ophaug, Unpublished data.

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oxygen-bomb reverse extraction technique (4). Fifteen milliliters of redistilled water was added to the oxygen bomb prior to combustion of the samples to act as a fluoride trap. Up to 0.3 ml of serum or urine was pipetted onto a 0.3-g pellet of filter paper pulp and fired without drying in the oxygen bomb. Larger volumes (up to 3 ml) of serum low in fluoride were pipetted onto the pellet and lyophilized prior to combustion. The method was slightly modified for the analysis of the feces in that 15 ml of total ionic strength activity buffer (TISAB, Orion Research, Inc.) was added to the oxygen bomb prior to firing instead of 15 ml of water. Blank and recovery samples were carried through the entire procedure.

Ultrafiltration studies. The pH of two 90-ml aliquots of human serum were adjusted to 7.4 by equilibration with a mixture of 95% air and 5% carbon dioxide. Seventy-five micrograms of nonionic fluorine (as perfluorooctanoic acid) was added to one aliquot and 1500 μg was added to the second. After 2 hr, 10 ml of the spiked serum was removed for determination of the initial concentration of perfluorooctanoic acid and the remaining serum containing perfluorooctanoic acid was transferred to an Amicon TCFIO ultrafiltration unit fitted with a Diaflo XM50 membrane which retains molecules greater than 50,000 daltons. The sample chamber was flushed with 95% air, 5% carbon dioxide, pressurized with nitrogen, and 15 ml of ultrafiltrate was collected. An ultrafiltrate was also prepared from pooled serum obtained from four rats 4.5 hr after receiving 2 mg of nonionic fluorine as perfluoroocatnoic acid by stomach intubation and from 75 ml of buffered (pH 7.4) isotonic saline to which 900 μg of nonionic fluorine had been added.

Statistics. Means were statistically compared by calculating Student's t value (11). A P value of less than 0.025 was chosen as indicating significance.

Results and discussion. Employing the oxygen bomb reverse extraction technique for determination of total fluorine, a recovery of 80 ± 1.0 SEM% was obtained for perfluorooctanoic acid. The recovery of fluoride from perfluorooctanoic acid was increased to 94 ± 1.5% when 15 ml of TISAB buffer, instead of water, was used as the fluoride trap inside the oxygen bomb. These recoveries with the closed, oxygen bomb system, compare favorably with a recovery of 21% obtained by open ashing procedures (7). The quantity of nonionic fluorine was calculated by subtracting the ionic fluoride from the total fluorine determined after ashing in the oxygen bomb and dividing by either 80 or 94%. The blank for the procedure employing a 0.3-g filter paper pulp pellet and 15 ml of redistilled water as a fluoride trap was 0.13 ± 0.020 μg. The modified procedure used to determine the total fluoride content of the feces had a blank of 0.40 ± 0.043 μg.

The results obtained in the ultrafiltration study of the binding of perfluorooctanoic acid in serum are given in Table I. The control experiment in which perfluorooctanoic acid was added to an isotonic saline solution buffered at pH 7.4 provides evidence that the ultrafiltration membrane binds very little perfluorooctanoic acid. Virtually all of the nonionic fluorine (as perfluorooctanoic acid) added to the saline solution was ultrafilterable. The addition of perfluorooctanoic acid to human serum, in amounts which elevated the nonionic fluorine to levels as high as 16 ppm, resulted in at least 99% of the added nonionic fluorine being bound to serum constituents and not being ultrafilterable. Serum harvested from rats 4.5 hr after the administration of a 2-mg dose of nonionic fluorine as perfluorooctanoic acid had a nonionic fluorine level in excess of 13 ppm and virtually all of this was bound to components in the serum and not ultrafilterable. The results of these experiments thus support the observations of Taves that the nonionic fluorine of human serum is not ultrafilterable (1).

The ionic and nonionic fluorine content of the serum at various times following the administration of the dose are shown in Fig. 1. Prior to intubation of perfluorooctanoic acid, the ionic and nonionic fluorine levels were 0.032 and 0.07 ppm, respectively. The presence of significant quantities of nonionic fluorine in rat and bovine serum has been reported previously by our labo-

TABLE I. FLUORIDE CONTENT OF SERUM, SERUM ULTRAFILTRATES, AND BUFFERED ISOTONIC SALINE BEFORE AND AFTER THE ADDITION OF PERFLUOROOCTANOIC ACID[a]

Specimen	Treatment	Specimen		Ultrafiltrate	
		Ionic	Total	Ionic	Total
Buffered saline (pH 7.4)	Perfluorooctanoic acid added	—	12.1	—	11.1
Human serum					
Aliquot 1	None	0.014	0.044	—	—
Aliquot 2	Perfluorooctanoic acid added	0.015	0.88	0.017	0.040
Aliquot 3	Perfluorooctanoic acid added	0.018	16.3	0.025	0.086
Rat serum					
1	Control serum[b]	0.032	0.11	—	—
2	Experimental serum[c]	0.013	13.2	0.016	0.12

[a] Fluoride contents are reported as ppm and are the mean of two determinations.
[b] Pooled serum from normal rats.
[c] Pooled serum from rats 4.5 hr after administration of a 2-mg dose of nonionic fluorine as perfluorooctane acid.

ratory (4, 5, 12, 13). Taves, however, has not found nonionic fluorine in the serum of rats and several other species of animals (14). The reason for this difference in results is not clear at the present time. Within 4.5 hr after the administration of the perfluorooctanoic acid the nonionic fluorine in the serum rose to 13.6 ppm. Despite the large increase (200-fold) in the nonionic fluorine level in the serum, the ionic fluoride level remained very low (0.03 ppm). The nonionic fluorine level in the serum decreased to 11.2 ppm at 8 hr and to 0.35 ppm at 24 hr. The level observed at 24 hr was still approximately seven times the baseline level. By 96 hr the mean level of nonionic fluoride in the serum had decreased to 0.08 ppm, a value that is not statistically different from that of the undosed animals. Throughout the entire 96 hr, the ionic fluoride level of the serum remained very low. On the basis of these data, it is clear that perfluorooctanoic acid is rapidly absorbed from the gastrointestinal tract and rapidly cleared from the serum. In view of the data from the ultrafiltration study, which showed that the perfluorooctanoic acid present in the serum of rats 4.5 hr after administration of the dose was tightly bound and not ultrafilterable, the rapid excretion of perfluorooctanoic acid in the urine *in vivo* would seem to be unlikely, as suggested by Guy *et al.* (7). However, since the *in vivo* data clearly showed a rapid removal of nonionic fluorine from the serum, the urine was analyzed for nonionic and ionic fluorine. The results are shown in Table II. Although the urine from the undosed animals contained no detectable nonionic fluorine,

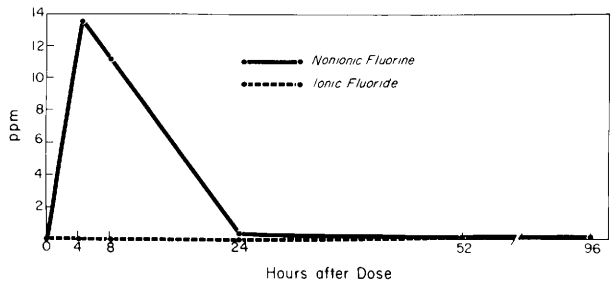

FIG. 1. Ionic and nonionic fluoride content of rat serum at various times following the administration of a 2-mg dose of nonionic fluorine as perfluorooctanoic acid.

TABLE II. URINARY EXCRETION OF IONIC AND NONIONIC FLUORIDE FOLLOWING ADMINISTRATION OF A 2-mg DOSE OF NONIONIC FLUORINE (AS PERFLUOROOCTANOIC ACID) TO RATS

Time (hr)	Ionic[a] (μg/hr)	Nonionic[a]	
		μg/hr	Accumulative percentage of dose
Baseline	1.73 ± 0.24[b] (4)	0	0
4.5	1.73 ± 0.22 (5)	166 ± 39.8 (5)	37 ± 9.0 (5)
8.0	2.36 ± 0.34 (5)	134 ± 33.6 (5)	61 ± 5.8 (5)
24	2.36 ± 0.54 (4)	19 ± 3.2 (4)	76 ± 2.7 (4)
52.5	1.83 ± 0.32 (11)	5 ± 1.2 (11)	81 ± 2.7 (11)
96	—[c]	5 ± 1.3 (4)	89 ± 2.6 (4)

[a] Excretion rates for ionic and nonionic fluoride were calculated as μg excreted/hr from the preceding time point. The baseline value was based on a 24-hr urine collection.

[b] Mean ± SEM (No. of animals).

[c] The ionic fluoride excretion rate for the 96-hr period is not given since these animals were fed a low fluoride diet.

within 4.5 hr after receiving the dose an average of 749 μg or 37% of the fluorine in the administered dose was recovered in the urine. The quantity of nonionic fluorine in the urine increased to 61% of the administered dose at 8 hr and by 24 hr 76% had been excreted in the urine. Between 24 and 96 hr of the experimental period the quantity of nonionic fluorine found in the urine increased to 89%. The rate of excretion of nonionic fluorine thus increased from 0 μg/hr in the undosed animals to 166 μg/hr 4.5 hr after receiving the dose of perfluorooctanoic acid. The rate of nonionic fluorine excretion rapidly decreased to 5 μg/hr over the interval from 52.5 to 96 hr (Table II). Thus, although perfluorooctanoic acid is apparently tightly bound to serum proteins, it is excreted rapidly into the urine. The mechanism by which this occurs is currently being investigated. The rate of excretion of ionic fluoride in the urine for the undosed animals, based on a 24-hr urine collection was found to be 1.73 μg F/hr. Although there is a tendency toward an increased ionic fluoride excretion in the dosed animals, in no instance was the ionic fluoride excretion rate statistically greater than that of the nondosed animals.

In an effort to account for the remaining nonionic fluorine, feces collected from dosed animals over 52.5- and 96-hr periods were analyzed for nonionic fluorine. The percentage of the administered dose of nonionic fluorine recovered in the urine and feces of animals after 52.5 and 96 hr is shown in Table III. After 52.5 hr a mean of 4.5% of the administered dose of nonionic fluorine was recovered in the feces. An additional 81.5% was present in the urine, bringing the total quantity of nonionic fluorine excreted in the feces and urine to 86%. By 96 hr, however, the quantity of nonionic fluorine excreted in the feces had increased to 14.3% and the entire dose of nonionic fluorine had been excreted in the urine and feces.

While certain fluorine-containing

TABLE III. PERCENTAGE OF THE ADMINISTERED DOSE OF NONIONIC FLUORINE RECOVERED IN THE URINE AND FECES AFTER 52.5 AND 96 hr

Time (hr)	Urine	Feces	Total
52.5	81.5 ± 3.9 (4)	4.5 ± 1.0 (4)	86.0 ± 3.3 (4)
96	89.3 ± 2.6 (4)	14.3 ± 4.1 (4)	103.5 ± 1.7 (4)

anesthetics such as methoxyflurane are metabolized with concurrent release of inorganic fluoride, this occurs without direct enzymatic attack on the carbon-fluorine bond. Instead, defluorination occurs as a result of the oxidation of the fluorine-containing carbon and the formation of an unstable molecule (15). Trifluoro-acetate, in which the end group contains three fluorine atoms, is generally regarded as inert (16). On the basis of the data presented here, perfluorooctanoic acid does not appear to be biologically oxidized and subsequently defluorinated.

Summary. The metabolic fate of perfluorooctanoic acid administered by stomach intubation to female rats was investigated. The nonionic fluorine level in the serum was increased 200-fold after administration of the dose but returned to baseline levels by 52.5 hr. Although perfluorooctanoic acid is rapidly absorbed and bound to nonultrafilterable components in the serum, the entire dose of nonionic fluorine was recovered in the urine and feces after 96 hr. Neither the ionic fluoride level in the serum nor the rate of ionic fluoride excretion in the urine was significantly altered by the administration of perfluorooctanoic acid. Although perfluorooctanoic acid has not been identified in the urine, the available data suggest that it has been excreted intact or in possibly conjugated form.

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