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RENAL FUNCTION AND FRACTIONAL CLEARANCES OF AMERICAN RIVER OTTERS (*LUTRA CANADENSIS*)

John P. Hoover[1] and Ronald D. Tyler[2]

ABSTRACT: The finely lobulated kidneys of American river otters (*Lutra canadensis*) are not visualized on plain abdominal radiographs. Similar values for blood urea nitrogen (BUN), creatinine, and uric acid were obtained on different analytical systems used in 1984 and 1985. The mean ± SD for measured plasma osmolalities (309.80 ± 8.86 mOsmol/kg) of otters in 1985 was significantly ($P < 0.01$) less than that of calculated serum osmolalities in the same 1985 specimens (321.61 ± 5.64 mOsmol/kg) and in 1984 specimens (322.20 ± 7.16 mOsmol/kg). Urine specific gravities and osmolalities were highly correlated ($r = 0.92$). On routine urinalysis, protein and bilirubin were frequent chemical findings, and urobilinogen was present in all urine samples. White and red blood cells and epithelial cells were frequent findings on urine microscopic examinations. *Proteus mirabilis* was cultured from four of four female otters with genitourinary infections. The mean ± SD creatinine values for paired serum and urine samples ($n = 13$) were serum creatinine (Scr) 0.66 ± 0.09 mg/dl and urine creatinine (Ucr) 186.9 ± 55.6 mg/dl. Corresponding values for serum electrolytes (Se) and urine electrolytes (Ue) yielded mean ± SD calculated renal fractional clearances (FC = Ue/Se × Scr/Ucr) of sodium $9.65 \pm 5.81 \times 10^{-4}$, potassium $4.15 \pm 2.01 \times 10^{-2}$, chloride $10.81 \pm 5.33 \times 10^{-4}$, calcium $4.52 \pm 4.46 \times 10^{-3}$, and phosphate $6.58 \pm 3.44 \times 10^{-3}$.

INTRODUCTION

River otters (*Lutra canadensis*) spend from 41 to 62% of their time engaged in foraging and feeding activities (Melquist and Hornocker, 1983) in a primarily aquatic environment. Virtually nothing is known about normal renal function of river otters or adaptations in renal physiology for their aquatic life. Twenty (10 and 10) river otters were wild-caught in Louisiana for release in Oklahoma in pilot reintroduction studies in 1984 and 1985. Clinical evaluations of these otters were performed prior to their release (Hoover et al., 1984; Hoover et al., 1985a). In this report, we present the combined 1984 and 1985 data on river otter renal function. Portions of these data have appeared previously in separate reports. Included in this report are comparisons of serum biochemical values obtained on different analytical systems, comparisons of calculated versus measured values for serum and plasma osmolalities, serum anion gaps, and renal fractional clearance values of electrolytes for American river otters.

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MATERIALS AND METHODS

River otters studied in 1984 (five females and five males) were maintained in cages indoors from days 1 through 5 as described previously (Hoover, 1984; Hoover et al., 1984) and then released on day 6 ($n = 10$). River otters studied in 1985 (five females and five males) were maintained both in outdoor pens from days 1 through 13 and days 19 through 25 and 29, and in cages indoors from days 14 through 18 as described previously (Hoover et al., 1985a; Hoover et al., 1985b) and then released on days 26 ($n = 6$) and 30 ($n = 1$). Otters were captured and held in Louisiana between 39 to 96 days prior to their transport to Oklahoma in 1984 and 1985 and were maintained on a diet consisting of raw nutria (*Myocastor coypu*) and alligator (*Alligator mississippiensis*) meat, dry dog chow, calf milk replacer, and cod liver oil. This diet was provided for otters during the 1984 study (Hoover, 1984; Hoover et al., 1984) and through day 3 of the 1985 study (Hoover et al., 1985a). On day 4 in 1985, a gradual change was made to an exotic feline diet (Nebraska Brand Feline Food, Central Nebraska

Packing Co., North Platte, Nebraska 69101, USA) (Hoover et al., 1985a). This change was completed by day 7, and the exotic feline diet was fed to otters for the balance of the 1985 study.

River otters were restrained chemically (immobilized) for clinical evaluations in 1984 and 1985 by intramuscular injection with a mixture of ketamine hydrochloride, xylazine hydrochloride, and acepromazine as described previously (Hoover, 1984, 1985; Hoover et al., 1984; Hoover et al., 1985a; Hoover and Jones, 1986). Otters were allowed water, but food was withheld overnight (at least 12 hr) prior to their immobilization on day 1 (n = 10) in 1984 and days 3 (n = 10), 15 (n = 8), and 24 (n = 8) in 1985 (two otters died, on days 5 and 7 respectively, in 1985). Clinical evaluations of immobilized river otters have been reported previously and included physical examinations, radiography (thoracic, abdominal, skull, and right forelimb views), complete blood counts (hemograms), serum biochemical analyses, urinalyses, and fecal and blood parasitologic examinations (Hoover et al., 1984; Hoover et al., 1985a); electrocardiography (Hoover, 1985; Hoover and Jones, 1986); and serologic determinations (Hoover et al., 1985b).

Blood specimens were obtained by jugular venipuncture from each otter on day 2 in 1984 (n = 10) and from each otter on days 3, 15, and 24 in 1985 (n = 26). Serum was separated from all clotted blood specimens within 1 hr and refrigerated (6 C). In 1985, anticoagulant (EDTA) blood specimens (n = 26) were refrigerated within 1 hr, and the plasma was removed after hemograms (Hoover et al., 1985a) had been performed. All serum biochemical analyses and plasma osmolalities (1985) were determined within 48 hr of specimen collection. In 1984, serum biochemical values (n = 10) for creatinine and uric acid were determined on a Technicon SMA II System (Technicon Corp., Tarrytown, New York 10591, USA) (Hoover et al., 1984). Serum biochemical values for blood urea nitrogen (BUN) of six specimens were determined on the Technicon SMA II System (Hoover et al., 1984), and four were determined on a Beckman BUN Analyzer 2 (Beckman Instruments, Inc., Brea, California 92621, USA) in 1984. In 1985, all biochemical values (n = 26) for BUN, creatinine, and uric acid were determined on an RA1000 System (Technicon Corp., Tarrytown, New York 10591, USA) (Hoover et al., 1985a). Serum osmolality (Sosm) estimates were calculated ($2Na^+ + 2K^+$ + glucose/18 + BUN/2.8) (Scott, 1982) from 1984 data (Hoover et al., 1984) and 1985 data. Plasma osmolalities (Posm) were measured by Advanced Osmometer Model 3W (Advanced Instruments, Inc., Needham Heights, Massachusetts 02194, USA) in 1985 (Hoover et al., 1985a). Serum electrolytes (Se) were measured on an RA1000 System in 1985 (Hoover et al., 1985a). Serum anion gaps were calculated ([Na^+ and K^+] − [Cl^- and HCO_3^-]) (Schaer, 1982) from 1984 data (Hoover et al., 1984) and by the RA1000 System in 1985 (Hoover et al., 1985a).

River otter urine specimens were obtained from clean-voided samples (n = 18) or aseptically by bladder catheterization (n = 2) of males and cystocentesis (n = 7) of females and refrigerated within 1 hr of collection. Urinalysis of the 12 specimens obtained from 10 otters in 1984 and 13 of 15 specimens obtained from nine of 10 otters in 1985 included urine chemistries measured by Ames N-Multistix and Ames Clini-Tek (Ames Division, Miles Laboratories, Inc., Elkhart, Indiana 46514, USA) and specific gravity measured by Total Solids Meter-Refractometer (American Optical Corp., Buffalo, New York 14215, USA) (Hoover et al., 1984; Hoover et al., 1985a). Urine microscopic examinations were performed on six specimens from six otters in 1984 and 11 specimens from eight otters in 1985. Urine chemical and specific gravity determinations were made within 8 hr, and microscopic examinations were performed within 48 hr of specimen collection. In 1985, urine osmolalities (Uosm) were measured for all 15 specimens by an Advanced Osmometer Model 3W (Hoover et al., 1985a); urine electrolytes (sodium, potassium, chloride, calcium, and phosphate) of 13 specimens collected on days 15 and 24 were measured on a Beckman System E4A (Beckman Instruments, Inc., Brea, California 92621, USA); and urine creatinine of the same 13 specimens were measured on an RA1000 System within 48 hr of specimen collection.

Specimens for microbial evaluations were obtained from four female otters (two in 1984 and two in 1985), with clinical findings indicating genitourinary infections (purulent vaginal discharge with leukocytosis) (Hoover et al., 1984; Hoover et al., 1985a) by urine cystocentesis and vaginal swabs. These specimens were refrigerated within 1 hr and inoculated into culture media within 24 hr. Both qualitative (identification) and quantitative aerobic microbial cultures were performed, and identified pathogens were tested for in vitro antimicrobial susceptibilities by the Bauer-Kirby procedure

(Bauer et al., 1966). In 1985, two affected otters were treated by intramuscular injections of gentamicin (Schering Corp., Kenilworth, New Jersey 07033, USA) at 8 mg/kg and procaine penicillin G (E. R. Squibb and Sons, Inc., Princeton, New Jersey 08540, USA) at 40,000 IU/kg given once daily for 6 days and then repeated in 1 wk (Hoover et al., 1985a). Urinalyses from these four otters were included in the data.

River otter renal fractional clearances were calculated from 13 paired urine and blood specimens from eight otters collected on days 15 and 24 in 1985 using:

$$\text{fraction clearance} = \frac{\frac{Ue \times \mathring{V}}{Se}}{\frac{Ucr \times \mathring{V}}{Scr}} = \frac{Ue}{Se} \times \frac{Scr}{Ucr}$$

where

Ue = urine electrolyte
Se = serum electrolyte
Scr = serum creatinine
Ucr = urine creatinine
$\mathring{V}$ = urine output (ml/min over 24-hr collection)

Each urine specimen was obtained within 15 min of blood specimen collection.

The means, standard deviations, and observed ranges are presented for the measured and calculated data. Where appropriate, means were compared by *t*-tests for independent samples with equal or unequal variances as determined by *F*-test or dependent paired samples (paired *t*-test); correlation coefficients were determined for the data by linear regression analysis; and data were checked for extreme values (Dixon and Massey, 1969). Minimum statistical significance was considered to be $P < 0.05$ unless stated otherwise.

RESULTS

River otter kidneys are finely lobulated and have no appreciable capsular fat (Fig. 1). The renal silhouettes were not visualized radiographically, even in river otters with abundant body fat and correspondingly good visceral detail (Figs. 2, 3).

Otter serum biochemical values for BUN, creatinine, and uric acid are presented in Table 1. Values obtained from the analytical systems compared were

FIGURE 1. The finely lobulated kidneys (intact capsules), ureters, and bladder of a female American river otter.

similar; however, small but statistically significant differences were found in creatinine. An extreme value ($P < 0.01$) for creatinine (2.1 mg/dl) and the corresponding BUN/creatinine (13) were omitted. The mean ± SD for calculated Sosm estimates was 322.20 ± 7.14 mOsml (range 313.5–333.6 mOsml) in 1984 ($n = 10$) and 321.61 ± 5.64 mOsml (range 311.9–336.2 mOsml) in 1985 ($n = 26$). The mean ± SD ($n = 26$) Posm measured by osmometer in 1985 was 309.80 ± 8.86 mOsml (range 292–327 mOsml). This was significantly ($P < 0.01$) lower by paired *t*-test than the mean calculated Sosm of the same specimens in 1985 and by *t*-test for independent samples with equal variances ($F = 1.54$, with 25 and 9 df) than the mean calculated Sosm in 1984. Calculated Sosm

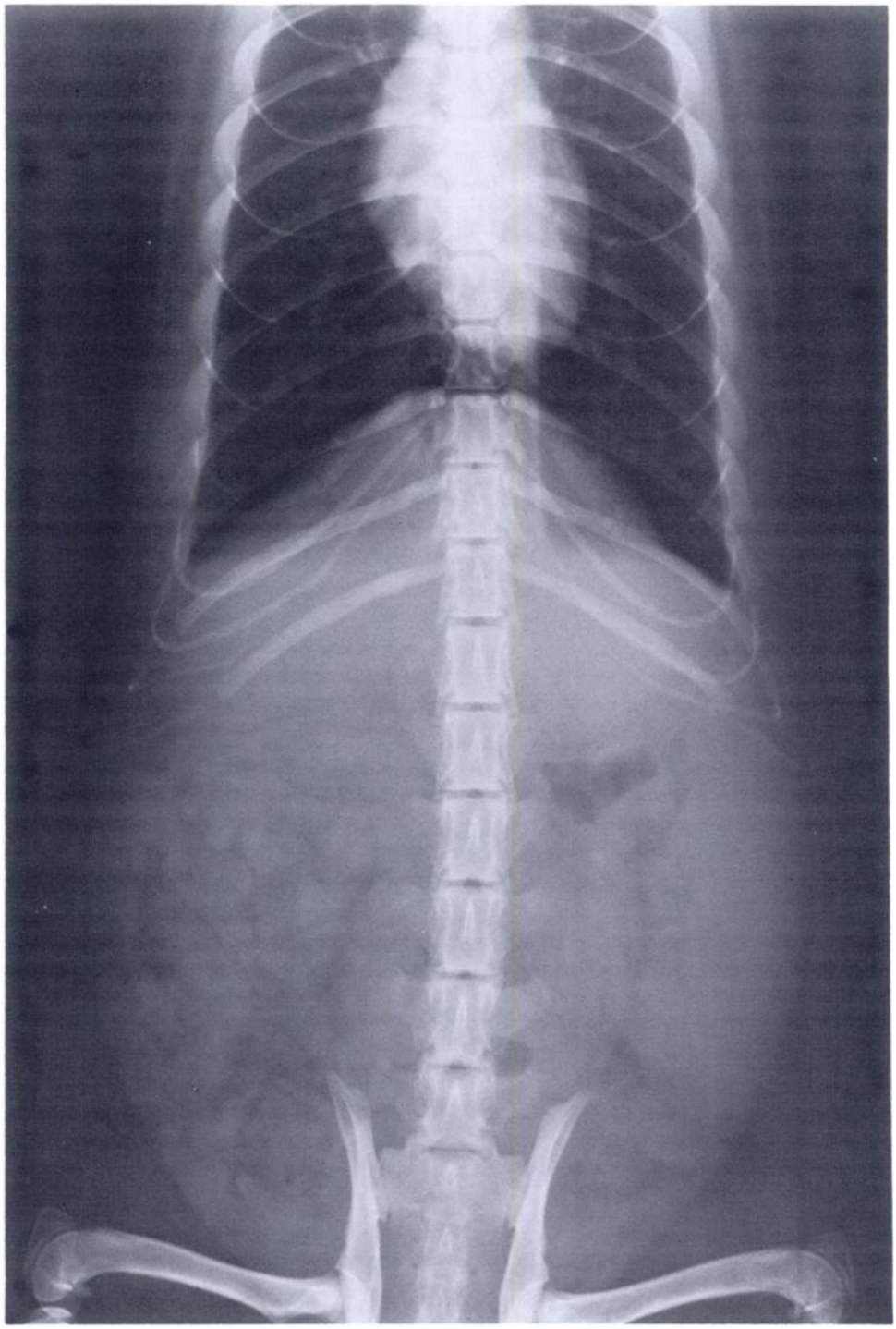

FIGURE 2. Ventrodorsal radiograph of a female American river otter (shown in Fig. 3). There is abundant subcutaneous fat with correspondingly good visceral detail (resolution). Silhouettes of the lobulated kidneys are not visualized radiographically.

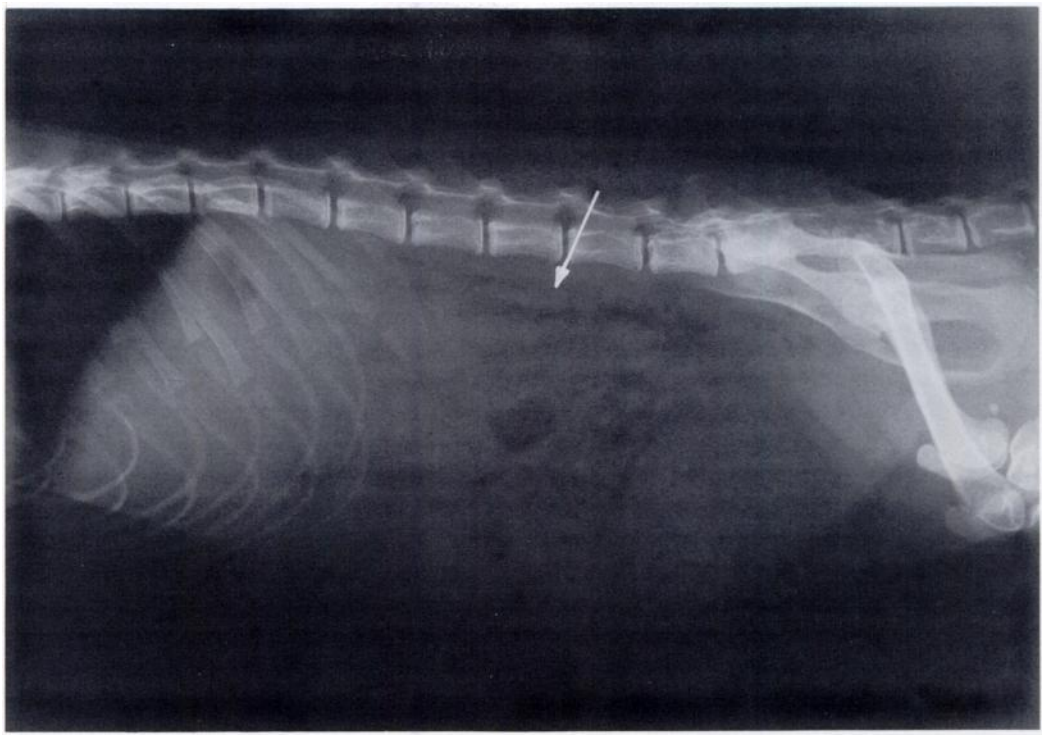

FIGURE 3. Right lateral radiograph of a female American river otter (shown in Fig. 2). There is abundant subcutaneous fat and retroperitoneal fat (arrow) with correspondingly good visceral detail (resolution). Silhouettes of the lobulated kidneys are not visualized radiographically.

estimates and measured Posm values for 1985 specimens were not highly correlated ($r = 0.41$, $n = 26$), and the variances were not equal ($F' = 2.46$, with 25 and 25 df). The mean ± SD ($n = 10$) serum anion gap of 15.1 ± 4.5 (range (9.7–20.6) calculated in 1984 was not significantly higher than the mean ± SD ($n = 26$) serum anion gap of 12.8 ± 4.5 (range 7–21) determined (calculated) by the RA1000 System in 1985 by t-test for independent samples with equal variances ($F' = 1.00$, with 9 and 25 df).

Urinalyses performed on specimens obtained from 10 otters (12 specimens) in 1984 were compared with those obtained from nine of 10 otters (15 specimens) in 1985 and are presented in Table 2. Urine specific gravity and Uosm of 1985 specimens were highly correlated ($r = 0.92$, $n = 13$). On day 3 in 1985, one female otter with a 1.020 specific gravity on urinalysis (clean-voided sample) had marked proteinuria (≥300 mg/dl) with hematuria (3+ with four to five leukocytes per high-power field) and bacteriuria (4+ and nitrite positive). This otter died day 7 and on histologic examination was found to have diffuse renal tubular necrosis, and *Salmonella anatum* was cultured from peritoneal fluid.

Two female otters in 1984 and another two in 1985 had clinical findings indicating genitourinary infections. *Proteus mirabilis* was cultured from urine (cystocentesis) and vaginal (swab) specimens of all four otters, a beta-hemolytic *Streptococcus* from one of the two otters in 1984, and a beta-hemolytic *Escherichia coli* from one of the two otters in 1985. Treat-

TABLE 1. Comparison of serum blood urea nitrogen, creatinine, and uric acid values of American river otters analyzed by different systems.

	1984 (10 otters)						1985 (10 otters)			Combined (20 otters)		
	Technicon SMA II			Beckman BUN Analyzer 2			RA1000 System					
	n[a]	$\bar{x} \pm$ SD	Range	n	$\bar{x} \pm$ SD	Range	n	$\bar{x} \pm$ SD	Range	n	$\bar{x} \pm$ SD	Range
BUN[b] (mg/dl)	6	28.2 ± 5.38	20–34	4	40.5 ± 17.82	14–51	26	32.8 ± 10.11	14–54	36	32.9 ± 10.72	14–54
Creatinine (mg/dl)	10	0.5[c] ± 0.05	0.5–0.6		—	—	25[d]	0.67[c] ± 0.10	0.6–0.9	35[d]	0.63 ± 0.11	0.5–0.9
BUN/creatinine	6	53.2 ± 11.2	40–68	4	75.8 ± 33.5	28–99	25[d]	48.4 ± 14.70	23–82	35[d]	52.4 ± 18.60	23–99
Uric acid (mg/dl)	10	2.07 ± 0.29	1.6–2.5		—	—	26	2.00 ± 0.72	0.5–3.4	36	2.01 ± 0.66	0.5–3.4

[a] n = number of blood specimens evaluated.
[b] BUN = blood urea nitrogen.
[c] Significantly different at $P < 0.01$ by t-test with unequal variances ($F' = 3.85$, with 24 and 9 df).
[d] Extreme value for creatinine (2.1 mg/dl) omitted, $P < 0.01$ (Dixon and Massey, 1969).

ment of the two otters in 1985 appeared efficacious in clearing these infections based on subsequent clinical evaluations (normal physical examination and hemogram and negative follow-up urine and vaginal cultures) of both animals. Subsequently, one of these otters died on day 27 from an unrelated retropharyngeal abscess (*Klebsiella pneumoniae*). Gross and histologic examination of this otter revealed no genitourinary infection.

The renal fractional clearances of electrolytes for river otters based on paired serum and urine specimens in 1985 are presented in Table 3. The mean ± SD (n = 13) for Ucr was 186.9 ± 55.6 mg/dl (range 108–304 mg/dl) and for Scr was 0.66 ± 0.09 mg/dl (range 0.6–0.9 mg/dl). The extreme value for Scr (2.1 mg/dl) and the corresponding value for Ucr (186 mg/dl) obtained from an otter released day 26 were omitted from the analysis. The mean ± SD (n = 14) for Uosm was 1,048.4 ± 231.5 mOsml (range 510–1,482 mOsml) and the corresponding mean ± SD (n = 14) for Posm was 314.2 ± 7.21 mOsml (range 304–327 mOsml), which resulted in a mean ± SD Uosm/Posm ratio of 3.33 ± 0.73 (range 1.68–4.75).

DISCUSSION

Blood nonprotein nitrogens consist primarily of urea and smaller amounts of creatinine, amino acids, uric acid, and ammonia (Medway et al., 1969), which are excreted by the mammalian kidney. Values obtained in this study (Table 1) may serve as a basis of comparison for values obtained on other American river otters.

Routine urinalyses performed in this study indicated that some protein and bilirubin are frequently found and small amounts of urobilinogen should be expected in urine specimens from American river otters (Table 2). Genitourinary infections in wild-caught captive female American river otters appeared common

TABLE 2. Comparison of urinalyses of American river otters obtained in 1984 and 1985.

	1984 (10 otters)				1985 (9 otters)				Combined (19 otters)			
	n[a]	$\bar{x}$ ± SD	% of samples	Range	n	$\bar{x}$ ± SD	% of samples	Range	n	$\bar{x}$ ± SD	% of samples	Range
pH	12	6.58 ± 1.10		5.0 to 9.0	13	5.88 ± 0.30		5.0 to 6.0	25	6.22 ± 0.85		5.0 to 9.0
Specific gravity	12	1.040 ± 0.017		1.018 to 1.077	13	1.030 ± 0.005		1.020 to 1.035	25	1.035 ± 0.013		1.018 to 1.077
Osmolality (mOsmol)		ND[b]		—	15	1,041 ± 224.9		510 to 1,482	15	1,041 ± 224.9		510 to 1,482
Chemistries												
Protein (mg/dl)	12		83	0 to ≥300	13		100	trace to ≥300	25		92	0 to ≥300
Glucose	12		0	—	13		0	—	25		0	—
Ketone	12		0	—	13		0	—	25		0	—
Bilirubin	12		75	0 to 3+	13		23	0 to 1+	25		48	0 to 3+
Blood	12		100	trace to 3+	13		85	0 to 3+	25		88	0 to 3+
Nitrite	12		0	—	13		8	neg to pos	25		4	neg to pos
Urobilinogen (mg/dl)	12		100	0.1	13		100	0.1	25		100	0.1
Microscopic (hpf[c])												
Casts (granular)	6		0	—	11		9	0 to rare	17		4	0 to rare
WBC	6		67	0 to occas[d]	11		100	occas to ≥8	17		83	0 to 8+
RBC	6		83	0 to TNTC[e]	11		91	0 to TNTC	17		87	0 to TNTC
Epithelial cells	6		100	occas	11		82	0 to occas	17		91	0 to occas
Crystals[f]	6		50	0 to occas	11		18	0 to occas	17		35	0 to occas
Bacteria[g]	6		50	0 to 4+	11		36	0 to 4+	17		43	0 to 4+

[a] n = number of urine specimens evaluated.
[b] ND = no determinations.
[c] hpf = high power field.
[d] occas = occasional (<1 per hpf).
[e] TNTC = too numerous to count.
[f] Crystals = triple phosphate > urate > oxalate.
[g] Bacteria: range, 0 (=none) to 4+.

TABLE 3. Renal fractional clearances of electrolytes by American river otters.[a]

	$\bar{x}$ ± SD	Range
Sodium		
Urine (mg/dl)	37.9 ± 19.2	14.4–70.5
Serum (mg/dl)	147.8 ± 3.2	141–154
FC[b] (10^{-4})	9.65 ± 5.81	3.21–24.59
Potassium		
Urine (mg/dl)	49.4 ± 20.5	10.0–74.6
Serum (mg/dl)	4.2 ± 0.5	3.6–5.0
FC (10^{-2})	4.15 ± 2.01	1.74–8.53
Chloride		
Urine (mg/dl)	33.1 ± 20.2	7.0–57.8
Serum (mg/dl)	113.3 ± 2.8	109–119
FC (10^{-4})	10.81 ± 5.33	3.66–18.06
Calcium		
Urine (mg/dl)	11.2 ± 7.5	4.0–28.0
Serum (mg/dl)	8.7 ± 0.7	7.5–9.7
FC (10^{-3})	4.52 ± 4.46	1.65–13.36
Phosphate		
Urine (mg/dl)	125.1 ± 71.9	6–199
Serum (mg/dl)	7.4 ± 1.3	4.7–8.8
FC (10^{-3})	6.58 ± 3.44	0.51–13.36

[a] Eight otters with paired urine and serum specimens, $n = 13$.
[b] FC = fractional clearance, or Ue/Se × Scr/Ucr, where Ue = urine electrolyte, Se = serum electrolyte, Scr = serum creatinine, and Ucr = urine creatinine.

(four of 10 females affected), and *Proteus mirabilis* appeared to be the most important microbial pathogen involved (four of four females affected). The high value for specific gravity (1.077) in Table 2 indicated that river otters are capable of highly concentrating urine.

The means for calculated Sosm values in 1984 and 1985 were essentially equal. However, there was a small (12 mOsm) but significant ($P < 0.01$) difference between calculated Sosm and measured Posm values, which suggested that the calculated values overestimated the plasma osmolalities.

A substance such as inulin, which is freely filtered by the glomerulus without being metabolized and is neither reabsorbed or secreted by the tubules, can be used to measure glomerular filtration rate (GFR) (Finco, 1980). Endogenous creatinine clearance, however, gives a reasonable estimate of GFR in species such as the dog (Finco, 1971). It is not known if river otters have some ability to secrete creatinine, which would result in slight overestimation of GFR:

$$\text{creatinine clearance} = \frac{\text{Ucr}}{\text{Scr}} \times \frac{\mathring{\text{V}}}{\text{BW}}$$

where

Ucr = urine creatinine (mg/dl)
Scr = serum creatinine (mg/dl)
$\mathring{\text{V}}$ = urine output (ml/min over 24-hr collection)
BW = body weight (kg)

The clearance ratio (fractional clearance) of a substance indicates how that substance is processed by the kidney (Koushanpour, 1976). If the substance is filtered through the glomerulus and then secreted by the tubules, the ratio of its clearance to that of inulin or creatinine will be >1, while, if it is filtered and then reabsorbed, this ratio will be <1 (Koushanpour, 1976). Urine 24-hr volume collections to determine $\mathring{\text{V}}$ necessary for calculation of creatinine clearances and inulin clearances to compare with creatinine were not performed in this study. However, Ucr and Scr determined for river otters provided the Scr/Ucr ratio necessary to calculate renal fractional clearances of electrolytes (FC = Ue/Se × Scr/Ucr) for river otters. The effects of immobilization agents on river otter urine and serum creatinine and electrolytes are not known, but the calculated fractional clearances of electrolytes measured were lower than those reported for normal dogs (without chemical restraint) by DiBartola et al. (1980) with the exception of calcium, which was higher. These lower clearances, if present in free-ranging river otters, may represent an adaptation to reduce loss of plasma solutes in a fresh-water

aquatic environment. There was no radiographic evidence of metabolic bone disease in these otters, which suggested that the higher calcium fractional clearance represented an excess of calcium in the diet (Nebraska Brand Feline Food) fed to otters or a difference in the calcium metabolism of otters.

When data on river otter urine output ($\mathring{V}$) and water consumption can be obtained, the osmolal clearance (volume required to excrete urine solutes in a solution isotonic with plasma) and the free-water clearance (excess solute free water) of otters can be determined:

$$\text{urine output } (\mathring{V}) = \text{Cosm} + C_{H_2O}$$

where

Cosm = osmolal clearance
C_{H_2O} = free-water clearance

and

$$\text{osmolal clearance (Cosm)} = \frac{\text{Uosm} \times \mathring{V}}{\text{Posm}}$$

where

Uosm = urine osmolality (mOsmol/kg)
Posm = plasma osmolality (mOsmol/kg)

The effects of immobilization agents on river otter urine and plasma (or serum) osmolalities was not known, but the Uosm/Posm ratio for river otters (3.33 ± 0.73 mOsm, range 1.68–4.75 mOsm) was less than that reported for normal dogs (6.13 ± 1.37 mOsm, range 2.71–8.13 mOsm) by DiBartola et al. (1980). This appeared to be the result of a lower Uosm (more dilute urine) for these otters than dogs (≤2,500 mOsm) (Osborne et al., 1983), while their Posm was similar to dogs (280–305 mOsm) (Kirk, 1983). This lower Uosm/Posm ratio, if present in free-ranging river otters, may represent an adaptation to increase free-water clearance (C_{H_2O}) in a freshwater aquatic environment.

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