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Anatomic and Physiologic Reference Values in Least Shrews (*Cryptotis parva*)

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Background and Purpose: **The least shrew is an established animal model for reproductive and pharmacologic research. Biologic reference data are needed to assess animal health status and provide a rationale for use of novel statistical programs to evaluate the effects of orally administered substances in toxicologic and pharmacologic studies.**

Methods: **Organ weights, blood biochemical and hematologic values, and food and water consumption data were collected from 50-day-old shrews after two weeks' consumption of a standard feline diet.**

Results: **In general, data correlated well with values reported for other mammalian species. Plasma phosphorus concentration was high. There was a significant difference in food and water consumption per gram of body weight between shrews at lower and upper (± 1 SD) weight ranges for the study. The 3.2-g animals consumed 27% more food per gram of body weight than did the 5.0-g animals.**

Conclusions: **The high phosphorus concentration was attributed to hemolysis resulting from the axillary cut method of blood sample collection. The small size of the shrew allowed demonstration of the Kleiber effect within a ± 1 SD weight range in a single species. The phenomenon necessitates the use of statistical methods other than the typical tests establishing the significance of the differences between the means of groups for oral toxicologic and pharmacologic studies.**

The least shrew (*Cryptotis parva)* was established as a laboratory animal in the mid-1960s (1). It has served as a research model for studies involving reproduction (2-4), behavioral pharmacology (5-8), toxicology (9), and factors associated with their diminutive size: fast heart rate (10), rapid aging (11), decreased numbers of somatosensory cortical fields (12), and adaptive features related to its response to the cold (13, 14). There is no biologic reference information available for this species. The objective of the study reported here was to provide investigators with a reference set of organ weight, hematologic, and blood biochemical values in a large, stable and defined population of least shrews. This information should be helpful for investigators and clinicians in assessing the health status of a shrew colony. Accurate food and water consumption records are lacking for any red-toothed shrews. These data are necessary to establishing proper statistical programs to evaluate toxicologic and pharmacologic feeding studies. Lastly, organ weights and blood reference values can serve as standards for preliminary studies evaluating wild shrews as bio-indicators for environmental contaminants.

Materials and Methods

Animals from the Kirksville College of Osteopathic Medicine's (KCOM) self-replenishing least shrew colony were the source for these studies. Shrews were individually housed in 27 × 24 × 20-cm polycarbonate cages in a climate-controlled cubicle (23 ± 2°C; 40 to 50% humidity; 12/12-h light/dark cycle). Each cage contained a wooden nest box filled with heat-treated (55°C; 12 h) grass clippings, watering device, food container with a feeding disk, and heat-treated (65°C; 24 h) sandy loam soil as litter. Ground feline diet and water were available ad libitum. All experiments involved animals that meet the established criteria regarding age (50 ± 10 days), body weight (4.2 ± 0.8 g), and compliance during the acclimation period (7 ± 2 days with no food spilled). The weight limits represent the mean weight plus or minus one SD of 50 randomly selected, appropriately aged shrews of each sex. Food and water consumption and body weight were recorded weekly. Cages were changed when the weekly measurements were recorded. All shrews in the study were individually identifiable by a microchip-based animal identification system (Bio Medic Data Systems, Inc., Maywood, N.J.), and records were maintained for each animal. Animals were deprived of food for 2 h, weighed, anesthetized with CO_2, and exsanguinated via axillary cuts after 14 days on the listed protocol. Blood samples were collected in microhematocrit tubes from the axillary cuts at the time of euthanasia. Pneumothorax was induced by a thoracic wall incision immediately after blood collection to ensure death. Collection of all blood samples occurred between 8 a.m. and noon. The hematologic tests and plasma collections were completed within 20 min of sacrifice. Plasma samples were refrigerated and evaluated within eight hours of collection. Hematologic values were determined, using a QBC Autoread Analyzer (Becton Dickinson Company, Sparks, Md.), and blood biochemical parameters were measured by use of a Model 91 Hitachi Clinical Analyzer (Hitachi, Ltd., Tokyo, Japan). Often plasma volume was not adequate to measure all of the clinical biochemical parameters. This required elimination of selected tests. Necropsies were performed on all shrews, with the following organs being weighed: spleen, liver, kidneys, female reproductive tract (ovaries, oviducts, uterus, and vagina to a line just proximal to the entrance of the

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urethra), testes, heart, interscapular brown adipose tissue (BAT), and brain. The greatest diameter also was recorded for the adrenal glands, Cowper's (bulbourethral) glands, ampullary gland, and uterine horns. Statistical analysis of quantitative data was accomplished by use of *t* tests or Mann-Whitney sum tests if the normality test failed, and by use of regression analysis (Sigma Stat, SPSS Inc., Chicago, Ill.). The KCOM animal care and use programs are fully approved by the AAALAC, International. All aspects of the studies received full Institutional Animal Care and Use Committee approval, and were conducted in accordance with the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* (15).

Results

Body weight and food and water consumption values were near expected (Table 1); however, there was considerable variation in the grams of food consumed per gram of body weight per day over the 1 ± SD weight range used in the study. A 3.4-g shrew consumed more than 27% more food per gram of body weight than did 5.0-g animals (Table 2). Water consumed per gram of body weight had patterns similar to that of food consumption, with 3.4-g shrews drinking more than 20% more water per gram of body weight per day than did 5.0-g animals (Table 3). Data for organ measurement and hematologic and blood biochemical results are presented in Tables 4–6. A *t* test was run for all parameters, comparing data according to sex. Significant differences were found for granulocyte count and chloride concentration. Females had a significantly ($P = 0.038$) higher granulocyte count, and chloride concentration in males was significantly ($P = 0.040$) higher, as determined by use of the Mann-Whitney sum test. The low chloride values in females were the result of retaining outliers in the statistical calculations protocol. Three female chloride readings were sufficiently low to have been excluded if outliers were removed.

Discussion

The sizable variation in captive shrews' weight was principally due to the large number of obese animals in the population. The quantal weight distribution curve was skewed to the right. Hence, the selection criteria for this study indicated a larger standard deviation and a mean value that was almost 0.2 g heavier than the actual mean value for the study groups. This large weight variation allowed documentation of the effects of mammalian size on food and water consumption within a single species. Small mammals have larger surface area relative to their mass, which increases the potential for heat loss. Therefore, mammalian metabolic rates increase, as mammals become smaller. Calorie consumption per unit time increases linearly at the 0.75 power when regressed against body weight (16). This phenomenon accounted for the disparity in food consumption per gram of body weight observed in this study. A 3.4-g shrew weighed more than 47% less than did a 5.0-g shrew, and consumed 27% more food per gram of body weight. Given the potential divergence in food consumed by members of a treatment group, statistical methods other than the typical tests establishing significance of the differences between the means of groups will be necessary for oral toxicologic and pharmacologic studies.

The only measurement that differed from values listed for other mammals was the high phosphorus (P) concentration. Several factors can account for this discrepancy. The least shrew (*Cryptotis parva*) probably has a high-normal P value. The only other shrew for which data are available (*Suncus murinus*) also has high P values (17). All shrews of this study were young, and young mammals have P values over twice as high as those of older animals (18). Calcium concentration was low in our shrews. Generally, high P values accompany low calcium values. In the nonfed state, P values increase (18). Our protocol called for shrews to be deprived of food for 2 h before blood collection. Finally, RBC hemolysis in the plasma sample greatly increases P concentration (18), and the axillary cut method of blood collection encourages hemolysis.

The higher granulocyte values for females appear to be valid results. A review of the data indicated readings with no outliers. This finding is not surprising, since there are several reports of sex dif-

Table 1. Body weight and food and water consumption for control animals in the 14-day study

Variable	Females	Males	Combined
Body weight (g)	4.06 ± 0.08 (47)	4.05 ± 0.07 (47)	4.06 ± 0.05 (94)
Food consumption (g/d)	1.49 ± 0.03 (47)	1.57 ± 0.04 (46)	1.53 ± 0.03 (93)
Water Consumption (g/d)	3.36 ± 0.08 (46)	3.36 ± 0.08 (46)	3.36 ± 0.06 (93)

Data are expressed as mean ± SEM (n).

Table 2. Food consumption (mg) per gram of body weight (wt) per day for control least shrews one standard deviation above and below mean study weight and the regression analysis from which these data were derived

Sex	3.4-g	5.0-g	Regression	*P*
Female	405	319	Food/g/d = 0.587 – (0.0536 × final wt)	<0.001
Male	424	332	Food/g/d = 0.620 – (0.0577 × final wt)	0.008
Combined sexes	414	324	Food/g/d = 0.605 – (0.0561 × final wt)	<0.001

Table 3. Water consumption (mg) per gram of body weight per day for control least shrews one standard deviation above and below mean study weight and the regression analysis from which these data were derived

Sex	3.4-g	5.0-g	Regression	*P*
Female	894	775	Water/g/d = 1.147 – (0.0744 × final wt)	0.029
Male	889	737	Water/g/d = 1.212 – (0.0950 × final wt)	0.049
Combined sexes	890	759	Water/g/d = 1.169 – (0.0820 × final wt)	0.004

Table 4. Organ measurements for control least shrews in the 14-day study

Organ	Females	Males	Combined
Spleen (mg)	11.4±0.61 (46)	11.5±0.68 (48)	11.5±0.50 (93)
(%)	0.28±0.01 (46)	0.28±0.02 (47)	0.28±0.01 (92)
Liver (mg)	226±4.29 (46)	232±4.78 (47)	228±3.12 (92)
(%)	5.59±0.01 (46)	5.76±0.08 (49)	5.68±0.06 (95)
Kidneys (mg)	83.1±1.85 (46)	79.5±1.73 (47)	81.3±1.27 (93)
(%)	2.06±0.05 (46)	1.97±0.04 (47)	2.01±0.03 (93)
Heart (mg)	45.9±0.97 (46)	45.5±1.22 (47)	45.7±0.78 (93)
(%)	1.14±0.02 (46)	1.13±0.02 (48)	1.13±0.02 (94)
BAT (mg)	289±0.02 (46)	247±0.02 (46)	268±0.02 (92)
(%)	6.79±0.48 (46)	5.93±0.37 (46)	6.36±0.31 (92)
Brain (mg)	118±0.19 (45)	114±0.21 (46)	116±0.14 (91)
(%)	2.93±0.06 (63)	2.86±0.06 (62)	2.90±0.04 (125)
Reproductive tract (mg) (%)	12.3±0.76 (45) 0.003±0.00 (33)	2.3±0.76 (45) NA	NA NA
Testes (mg)	44.7±0.26 (46)	44.7±0.26 (46)	NA
(%)	1.11±0.07 (46)	1.11±0.07 (46)	NA
Adrenal gland (diameter [mm])	1.05±0.03 (46)	0.95±0.02 (44)	1.00±0.02 (90)
Uterine horn (diameter [mm])	0.66±0.01 (46)	NA	NA
Cowper's gland (diameter [mm])	NA	2.05±0.06 (47)	NA
Ampullary gland (diameter [mm])	NA	1.60±0.04 (47)	NA

BAT = interscapular brown adipose tissue; NA = Not applicable.
Data are expressed as mean ± SEM (n).

Table 5. Hematologic parameters for control least shrews in the 14-day study

Variable	Females	Males	Combined sexes
Hematocrit (%)	54.09±0.51 (42)	54.54±0.65 (46)	54.33±0.42 (88)
Hemoglobin (g/dl)	17.80±0.15 (40)	17.61±0.41 (43)	17.70±0.22 (83)
WBC (10^9/L)	2.23±0.12 (42)	1.98±0.10 (46)	2.10±0.08 (88)
Granulocyte (10^9/L, %)	1.11±0.07 (42), 51.38±2.09 (42)	0.92±0.06 (46), 47.11±1.97 (46)	1.01±0.05 (88), 49.15±1.44 (88)
Lymphoctye/ monocyte (10^9/L, %)	1.21±0.09 (42), 48.62±2.09 (42)	1.04±0.13 (46), 52.80±2.01 (45)	1.08±0.08 (88), 50.78±1.46 (87)
Platelet (10^9/L)	456.38±15.93 (42)	477.11±20.20 (46)	467.22±12.99 (88)

Data are expressed as mean ± SEM (n).

Table 6. Blood biochemical parameters for control least shrews in the 14-day study

Variable	Female	Male	Combined sexes
Glucose (mg/dl)	123.84±6.58 (61)	136.57±6.04 (48)	127.96±4.60 (109)
Sodium (mmol/L)	130.96±8.67 (27)	152.84±6.30 (14)	138.43±6.27 (41)
Potassium (mmol/L)	6.79±0.34 (27)	7.58±0.46 (14)	7.06±0.28 (41)
Chloride (mmol/L)	103.73±5.32 (26)	118.85±4.70 (14)	109.02±3.96 (40)
Calcium (mg/dl)	7.25±0.38 (22)	6.72±0.33 (10)	7.18±0.27 (31)
Phosphorus (mg/dl)	16.86±1.04 (18)	18.52±1.54 (16)	17.59±0.89 (33)
BUN (mg/dl)	90.70±4.36 (21)	90.88±2.65 (20)	87.50±2.74 (32)
Creatinine (mg/dl)	0.23±0.01 (18)	0.21±0.01 (13)	0.22±0.01 (31)
Total protein (g/dl)	5.12±0.11 (27)	4.99±0.11 (24)	5.03±0.07 (50)
Albumin (g/dl)	4.58±0.11 (18)	4.50±0.09 (18)	4.54±0.07 (36)
Bilirubin (mg/dl)	0.26±0.02 (16)	0.30±0.03 (16)	0.28±0.02 (32)
Alkaline phosphatase (U/L)	74.65±6.82 (28)	73.09±6.47 (25)	73.91±4.68 (53)
ALT (U/L)	147.30±11.39 (37)	130.40±10.07 (38)	139.00±7.70 (74)
Cholesterol (mg/dl)	34.70±2.66 (20)	38.57±2.43 (22)	36.75±1.84 (41)
Triglycerides (mg/dl)	76.75±5.62 (24)	75.24±5.74 (25)	76.07±4.06 (48)

ALT = Alanine transaminase.
Data are expressed as mean ± SEM (n).

ferences in mammalian species' white blood cell counts. Neutrophil counts are higher in female cats and chimpanzees (19, 20).

Literature indicates a proclivity of shrews to bioaccumulate toxins. The tissue collected from shrews living in contaminated terrestrial ecosystems has a manifold higher concentration of heavy metals and organic toxins than do the tissues of sympatric rodent species (21-23). Some of the parameters reported to indicate toxicosis in laboratory rodents are leukocytosis, anemia, splenomegaly, and hypercholesterolemia (24-26). The shrew spleen can serve as an indicator for all of the listed anomalies. It is a primary hematopoietic organ, including erythropoiesis (27). Splenomegalia also develops in shrews due to the phagocytosis of lipids, resulting in accumulation of macrophage-derived foam cells (28). One other organ deserves special mention. Bulbourethral glands are of value in assessing reproductive status. Their retroperitoneal location retards the rapid postmortem autolysis commonly observed in the viscus of shrews. This is of particular importance in evaluating decomposing wild shrew carcasses.

The contents of this report provide reference information for an extant mammal that offers great potential as a model for reproductive and pharmacologic studies. Given the shrews' capacity to bioaccumulate environmental contaminates, our findings could provide standards for preliminary evaluations for polluted terrestrial ecosystems, using shrews as models. The reported variations in food consumption per unit of body weight, on the basis of shrew size, necessitates use of statistical programs based on the evaluation of the linear or curvilinear nature of the data in oral dosing studies. Additional reference values for sequential age categories into senescence could provide biomarkers for age-related changes in this rapid-living, short-lived species.

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