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Hematologic and Biochemical Reference Intervals and Urinary Test Results for Wild-caught Adult Southern Giant Pouched Rats (*Cricetomys ansorgei*)

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Southern giant pouched rats (*Cricetomys ansorgei*) are muroid rodents native to subSaharan Africa. They are increasingly used as service animals because of their keen sense of smell and are primarily known for clearing minefields in Africa. The objectives of this study were to determine hematologic and biochemical reference intervals from clinically healthy wild-caught captive adult rats, to describe the cytochemical staining reactions of peripheral blood leukocytes, and to document urinalysis findings. Blood samples were collected from the coccygeal artery of 60 isoflurane-anesthetized rats (36 males and 24 females) and analyzed with automated hematologic and biochemical analyzers; manual differential cell counts were performed on modified Wright–stained blood smears. Urine was collected by cystocentesis, and dipsticks were analyzed on a urine analyzer, with visual examination of unstained sediments. Samples from a male rat with chronic renal disease were excluded from analysis. Reference intervals were determined according to guidelines established by the American Society of Veterinary Clinical Pathology. Lymphocytes were the dominant leukocyte in peripheral blood and granular lymphocytes were identified in most animals. Male rats had significantly higher RBC, absolute reticulocyte counts, and MCV than did female rats. Minor sex-associated differences in urea nitrogen concentration and GGT activity were noted. Leukocytes showed unique cytochemical staining characteristics. Small amounts of protein and bilirubin were found in the urine of rats of both sexes and of female rats, respectively, particularly in concentrated urine. These results will provide benchmarks for determining health status and identifying disease in this species of rat.

Abbreviations: ANBE, α-naphthyl butyrate esterase; ASVCP, American Society of Veterinary Clinical Pathology; CAE, chloroacetate esterase; HPF, high-power field; SGP, southern giant pouched

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African giant pouched rats belong to the family *Nesomyidae* and genus *Cricetomys*, and include multiple species of muroid rodents that are distributed across subSaharan Africa. Their name refers to their large cheek pouches, which are used to store food. The rats came to prominence when they were trained by a nongovernmental organization, APOPO ('AntiPersonnel Landmine Product Development'), to detect landmines and thus earned the nickname 'HeroRATs'.[31,32] These rodents have also been used for the detection of pathogens such as tuberculosis in sputum samples.[31,43] and can be trained as 'sniffer' animals to locate buried survivors at disaster sites.[20]

To our knowledge, the only available publication on these rats is a single limited report of selected biochemical results for 8 (4 male and 4 female) Gambian rats.[28] This lack of published clinical pathologic data for species of giant pouched rats may hinder health assessment and disease identification in these animals. The objectives of our current study were to establish hematologic and biochemical reference intervals for wild-caught clinically healthy adult Southern giant pouched (SGP) rats (*C. ansorgei*), describe cytochemical staining properties of leukocytes, and report urinalysis findings.

Materials and Methods

Animals. Wild SGP rats were caught at a field site (Morogoro, Tanzania) and transported to our institution for a long-term research project unrelated to this study. At 3 to 5 d after arrival in the United States, an oral swab and nonanticoagulated blood samples were collected from each animal under isoflurane anesthesia and sent to the Centers for Disease Control and Prevention (Atlanta, GA) to test for monkeypox viral DNA (real-time PCR analysis) and serum orthopoxvirus antibodies (ELISA), respectively. All samples were negative for both tests. The rats were quarantined for 3 mo. One female rat was noted on arrival to have a compound tibial fracture with a mild purulent discharge. This rat was treated, and the injury was completely healed at the time of sample collection for this study. A tail injury occurred in a second female rat at 4 wk into the quarantine period; this

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injury appeared to have healed at the time of sample collection for this study (7 to 8 wk later).

Fecal flotations were performed on the rats at the beginning of the quarantine period, and multiple endoparasites (nematodes and cestodes) were found. In addition, a few animals had earwigs (suborder Hemimerina) on their dorsal fur. These animals were treated with multiple doses of fenbendazole, moxidectin, and praziquantel during quarantine. Fecal flotation and fur test results were negative at a follow-up examination performed at 2 to 3 wk before starting this study.

The animals were individually housed at our AAALAC-accredited facility in stainless steel cages (24 in. long × 24 in. wide × 15.5 in. high; Hoeltge, Cincinnati, OH) with grid cage bottom. A stainless-steel mailbox-shaped hut was provided in each cage for the animals to hide in and sleep in. Newspaper or packing papers were torn and provided weekly as nesting material. Treated water (reverse osmosis–purified, carbon-filtered, and chlorinated) via automatic watering systems (automated watering valves) and chow (2019 Teklad global 19% protein-extruded diet, Envigo, Indianapolis, IN) in a J-feeder were provided without restriction. A variety of enrichment was provided regularly, such as wood gnawing blocks (2 in. × 2 in. × 2 in.), washed and cut fresh fruits and vegetables, and toys filled with pumpkin or sunflower seeds or peanut butter. The room was maintained at 22 to 24 °C, 35% to 50% relative humidity, and 12:12-h light:dark cycles. The airflow in the room was set at 12 air changes per hour and was negative to the corridor. All animal handling and procedures were done at or above ABSL2 practices to mitigate personnel exposure to any pathogens. This study was approved by the IACUC of Cornell University. We adhere to the following guidelines: *AALAS Position Statement on the Humane Care and Use of Laboratory Animals*,[1] the *Guide for the Care and Use of Laboratory Animals* (Institute for Laboratory Animal Research, National Academies Press, Washington, DC),[16] the Animal Welfare Act,[40] and the *United States Public Health Service Policy on the Humane Care and Use of Laboratory Animals* (Office of Laboratory Animal Welfare, NIH).[39]

Laboratory animal veterinarians experienced in working with this species performed complete physical examinations on all rats prior to sample collection, and all animals were deemed healthy. All rodents were estimated to be older than 1 y, based on body length.[34] Rats were not included in the study if they were deemed clinically unhealthy, had undergone medical or surgical procedures, had received medications during the 30 d period before sample collection, or were being used for breeding or research studies. Individual animals were excluded from the study if the initial assessment of laboratory results indicated probable underlying disease.

Table 1. Observed total error (TE_{obs}) for the ADVIA 2120 hematology analyzer for the hematologic measurands, with allowable total error (TE_a) as defined by the ASVCP.[24]

Variable	Low control TE_{obs} (%)	Normal control TE_{obs} (%)	High control TE_{obs} (%)	ASVCP TE_a (%)
Hct	2	3	4	10
HB	2	2	2	10
RBC	2	3	2	10
MCV	2	2	3	7
MCHC	3	4	4	10
RETIC*	9	9	21	20
WBC	4	4	5	15
PLAT	8	7	5	20

The TE_{obs} was calculated from the following formula: TE_{obs} = (2×analyzer CV) + bias for each level of control, where bias is the difference between the mean analyzer result and the manufacturer's designated control mean. The TE_{obs} for the 3 levels of controls was measured over 2 wk.
* The low and high controls had the highest (7.8%) and lowest (0.9%) means, respectively.
HB: Hemoglobin, RETIC: Reticulocyte percentage, PLAT: Platelet count

Blood and urine sample collection. Rats were sampled over a 6-wk period from January to mid-February 2016. Strict adherence to collection and handling protocols were followed for all animals. These animals are nocturnal species, and room lights were set on-off at 0600 and 1800 h. The sample collections were performed between 0900 and 1300 h. Blood was collected from unfasted, clinically healthy animals under isoflurane anesthesia. Each animal was transported from the home cage to the induction station by using a rat microisolation cage that had been modified for use an induction cage. Anesthesia was induced by using 5% isoflurane and maintained with 2% to 3% isoflurane. At approximately 5 min after induction, blood (2 mL) was collected from the midventral tail artery into a 3-mL syringe (Covidien, Mansfield, MA) by using a 23-gauge needle (Monoject, Covidien). Blood was then placed into EDTA and lithium-heparin vacuum microtubes (Becton Dickenson, Franklin Lakes, NJ) for hematologic and biochemical analysis, respectively. Based on our experience, tail arterial phlebotomy was a better technique than tail venous phlebotomy, which led to excessive sample clotting. Urine was collected into a 3-mL syringe (Covidien) via cystocentesis using a 23-gauge needle (Monoject, Covidien) from rats with readily palpable bladders. Urine was then transferred into a sterile red-top tube (Becton Dickenson) for laboratory submission.

Samples were submitted within 4 h of collection to the Clinical Pathology laboratory of the Animal Health and Diagnostic Center at Cornell University. Samples that were not taken to the laboratory immediately after collection were refrigerated at 4 to 8 °C until transfer. Samples were analyzed within 1 to 2 h of submission after being allowed to come to room temperature. The laboratory has an established quality-control program and participates in external proficiency testing with the College of American Pathologists and Veterinary Laboratory Association.

Hematologic analysis. EDTA-anticoagulated blood samples were assessed for clots by rimming the tube with wooden applicator sticks. Samples with identified clots ($n = 5$) were excluded from analysis, and blood was recollected from the same animals into an EDTA anticoagulant tube during the 6-wk study period, 1 to 2 wk later, according to the original protocols. The blood was analyzed by using an automated hematology analyzer (Advia 2120, Siemens Healthcare Diagnostics, Tarrytown, NJ), on which 3 levels of controls (Siemens Healthcare Diagnostics) were run daily on each business day. The observed total error for the controls was within allowable total error, as defined by the American Society of Veterinary Clinical Pathology,[24] except for the high control for reticulocyte percentage, which was barely outside the allowable total error and had the lowest mean control value (0.9%; Table 1). The automated analyzer generated the following results: RBC count, Hgb, Hct, MCV, MCH, MCHC, RBC distribution width, WBC count, platelet count, MPV, and percentage and absolute reticulocyte counts.

Smears were prepared from the EDTA-anticoagulated blood and stained by using a modified Wright stain (Hema-tek 1000, Siemens Healthcare Diagnostics) for blood smear examination, including a manual leukocyte differential count (performed by a single board-certified clinical pathologist [LEB]). Absolute leukocyte counts were calculated from the automated WBC

count and manual differential percentages. The numbers of nucleated RBC per 100 WBC and of granular lymphocytes as a percentage of lymphocytes were calculated. Microhematocrit tubes (Fisherbrand microhematocrit capillary tubes, Thermo Fisher Scientific, Waltham, MA) filled with EDTA-treated blood were spun in a microhematocrit centrifuge (Thermo IEC Micro CL17 centrifuge, Thermo Fisher Scientific) for 3 min at 11,700 × *g* at room temperature; total protein concentration was measured on the resulting EDTA-treated plasma by using a temperature-compensated hand-held refractometer (Reichert, Depew, NY). The plasma in the centrifuged microhematocrit tubes was visually inspected for hemolysis, lipemia, and icterus.

Biochemical analysis. Lithium heparin–anticoagulated whole blood samples were centrifuged (Sorvall ST16, Thermo Fisher Scientific) at 3000 × *g* for 10 min at room temperature. Biochemical analysis of analytes was performed by using by a Hitachi P Modular analyzer (Roche Diagnostics, Indianapolis, IN) for all analytes except lipase, which was measured by using a Cobas 501 (Roche Diagnostics), and (for most analytes) the manufacturer's reagents (Table 2). Two levels of manufacturer's controls were run daily on each business day. The observed total error for the controls was within the allowable total error as defined by the ASVCP[13] (Table 3). The following variables were reported: sodium, potassium, chloride, bicarbonate, urea nitrogen, creatinine, calcium, phosphorus, magnesium, total protein, albumin, glucose, total bilirubin, direct bilirubin, iron, cholesterol, triglycerides and nonesterified fatty acids concentrations and AST, ALT, GGT, ALP, creatine kinase, amylase, and lipase activities. Total iron-binding capacity was calculated from the sum of a direct measurement of unsaturated iron binding capacity and iron. The following variables were calculated also: sodium:potassium ratio, anion gap, globulin concentration (i.e., total protein – albumin), albumin:globulin ratio, indirect bilirubin (i.e., total bilirubin – direct bilirubin), and percentage of transferrin saturation with iron (saturation = [iron ÷ total iron-binding capacity] × 100%). The analyzer also provides semiquantitative measures (called 'indices') of the degree of icterus, hemolysis, and lipemia, which are based on bichromatic wavelength measurements (according to the manufacturer's instrument manual).

Table 2. Methods used for measurement of biochemical analytes with the Hitachi P Modular (most analytes) and Cobas 501 (lipase only) in lithium-heparinized plasma from Southern giant pouched rats (*Cricetomys ansorgei*).

Analyte	Method
Albumin	Bromcresol green dye-binding
ALP	*p*-nitrophenylphosphate cleavage
Amylase	Saccharogenic cleavage
ALT	L-alanine substrate, with added pyridoxal 5′-phosphate
AST	L-aspartate substrate, with added pyridoxal 5′-phosphate
Bicarbonate	Phosphoenolpyruvate carboxylase-based
Bilirubin, total	Diazo method (diazonium ion)
Bilirubin, direct	Diazo method (diazotized sulfanilic acid)
Calcium	o-cresolphthalein
Cholesterol	Cholesterol esterase
Creatine kinase	Creatine phosphate cleavage
Creatinine	Jaffe (picric acid)
Electrolytes (sodium, potassium, chloride)	Ion-selective electrodes
GGT	L-γ-glutamyl-3-carboxy-4-nitroanilide substrate
Glucose	Hexokinase
Iron	Ferrozine
Lipase	1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methyl-resorufin) ester substrate (DGGR)
Magnesium	Xylidyl blue binding
Nonesterified fatty acids*	Acyl-coA synthetase
Phosphate	Ammonium molybdate
Total protein	Biuret
Triglycerides	Lipoprotein lipase
Unsaturated iron-binding capacity	Ferrozine with excess iron
Urea nitrogen	Urease kinetic

Manufacturer reagents were used for the analytes, unless otherwise indicated.
* NEFA-HR (2), Wako Diagnostics, Mountain View, CA

Cytochemical staining of leukocytes. Cytochemical staining of leukocytes was performed on air-fixed blood films from 2 male and 2 female rats within 1 wk of sample collection. Staining for ALP, α-naphthyl butyrate esterase (ANBE), chloroacetate esterase (CAE), and myeloperoxidase was performed by using commercial kits according to manufacturer's procedures (ALP, Product Number 85L2; ANBE, Product Number 181B; CAE, Procedure Number 91C; Myeloperoxidase, Product Number 390A; Sigma–Aldrich, St Louis, MO). Canine blood was used as a positive control for ANBE (monocytes, platelets), CAE (neutrophils) and myeloperoxidase (neutrophils). ALP staining was followed by a 1-min counterstain with Mayer hematoxylin, using a smear of equine blood as a positive control (neutrophils). The intensity, localization, and pattern of staining reactions was graded by a single observer (TS) from representative photomicrographs taken of the stained smears for all 4 animals. Intensity was graded as weak (1+), moderate (2+), or strong (3+). Staining localization was identified as being in granules or in the cytoplasm, with the pattern of staining in the cytoplasm further distinguished as focal, multifocal, or diffuse.

Urinalysis. Urine volume, color, and clarity were recorded. Urine specific gravity was assessed via refractometry (Reichert-Vet 360 TS, temperature-compensated, Reichert Analytical Instruments) on uncentrifuged urine. Urine chemical analysis was performed on uncentrifuged urine by using test strips (Multistix, Siemens Healthcare Diagnostics), which were read on an automated urine analyzer (Clinitek Advantus, Siemens Healthcare Diagnostics). The following results were reported from the analyzer: pH; semiquantitative concentrations for protein (0 or negative, 0.15 g/L or trace; 0.3 g/L or 1+; 1 g/L or 2+; and 3 g/L or 3+), glucose (negative, 5.6 mmol/L or trace; 14 mmol/L or small; 28 mmol/L or moderate; and 56 mmol/L or large), ketones (negative, trace, small, moderate, and large), and categorical amounts of blood (negative, trace, small, moderate, large) or bilirubin (negative, small, moderate, large). The analyzer did not yield readings for urine pH above 9.0. Urine samples with positive dipstick reactions for bilirubin were re-tested for bilirubin immediately after dipstick analysis by using reagent tablets (Ictotest, Siemens Healthcare Diagnostics) to verify the positive result. Urine was then centrifuged for 3 min at 202 × *g* (Heraeus, Thermo Fisher Scientific) and the supernatant was removed. A drop of the urine sediment was placed on a glass slide, cover-slipped, and examined by light and phase microscopy for the presence of WBC, RBC, crystals, casts, microorganisms, epithelial cells, debris, fat, and spermatozoa;

Table 3. Observed total error (TE_{obs}) for the Hitachi P Modular (most measurands) and Cobas 501 (lipase) biochemical analyzers for the measurands, with allowable total error (TE_a) as defined by the ASVCP.[13] The TE_{obs} was calculated from the following formula: TE_{obs} = (2xanalyzer CV) + bias for each level of control, where the bias is the difference between the mean analyzer result and the manufacturer's designated control mean. The low and high control TE_{obs} were measured over 1 mo.

Analyte	Low control TE_{obs} (%)	High control TE_{obs} (%)	ASVCP TE_a (%)
Albumin	5	6	15
ALP	10	12	20
Amylase	4	3	25
ALT	6	3	25
AST	7	3	30
Bicarbonate	11	8	15
Bilirubin, total	11	4	30
Bilirubin, direct	3	7	—
Calcium	3	4	10
Cholesterol	4	3	20
Chloride*	3-4	2-3	5
Creatine kinase	5	3	30
Creatinine	9	6	20
GGT	4	5	20
Glucose	5	4	20
Iron	6	4	30
Lipase	7	8	—
Magnesium	6	4	15
Nonesterified fatty acids**	7	—	—
Phosphate	11	6	15
Potassium*	4-5	2-2	5
Sodium*	2-2	1-2	5
Total protein	5	2	10
Triglycerides	6	4	25
Unsaturated iron-binding capacity	17	19	—
Urea nitrogen	7	7	12

* Two electrolyte units were available on the Hitachi P Modular and the results reflect the range of values for both units.
** Only one level of control available.
-: Not available.

these were visually quantified per high-power field (/HPF; 40× objective) for WBC and RBC and per low- (10× objective) and high-power field for the remaining constituents (very few, moderate, and many or slight, moderate and large, depending on the constituent).

Statistical analysis and determination of reference intervals. Before reference intervals were determined, any EDTA-anticoagulated blood samples that were icteric, moderately or severely hemolyzed, or lipemic on visual inspection of the plasma were excluded from the study. Similarly, any lithium heparin-anticoagulated plasma with icterus (icteric index > 0) or moderate to severe hemolysis (hemolytic index > 100 units) or lipemia (lipemic index > 60 units), as defined in our laboratory,[8] was excluded from the study. If an animal had clinical pathologic data consistent with subclinical disease, all samples from that rat were excluded.

Reference intervals for hematologic and biochemical results were established according to the guidelines of the ASVCP.[11] Intervals were generated by using Reference Value Advisor,[12] an Excel add-in (Microsoft, Redmond, WA). The program determines Gaussian distribution by using the Anderson–Darling goodness-of-fit test and identifies potential outliers by using Dixon range statistics and Tukey interquartile fences. The data were also visually inspected for outliers, with preference given to data retention. Nonparametric methods were used to establish upper (97.5th percentile) and lower (2.5th percentile) reference limits. Because fewer than 120 reference subjects were used, the program determined 90% CI on these reference limits by using a bootstrap method. Untransformed standard gaussian and Box–Cox transformed standard data were also performed when statistically appropriate.

The hematologic and biochemical data were partitioned by sex and described as mean ± 1 SD for Gaussian data or as median and range for nonGaussian data. Mean and median results for each analyte were compared between sexes by using the Student *t*-test or Mann–Whitney *U* test, respectively, after outliers (determined from the sex-combined reference intervals) had been removed. An analyte was considered normally distributed based on equivalent results with the D'Agostino and Pearson and Shapiro–Wilk tests, which were performed simultaneously with statistical software (Prism 7 for Mac OS, version 7.0c, GraphPad Software, San Diego, CA).

For urinalysis, numerical or ordinal data were represented as mean ± 1 SD with range or as median with range for Gaussian or nonGaussian data, respectively. Normality was determined using D'Agostino and Pearson and Shapiro–Wilk normality tests (Prism 7 for Mac OS). Differences in numerical and ordinal data (urine specific gravity, protein concentration, and pH) were compared between sexes as described above. For the urine pH, values greater than 9.0 were designated as 9.5 for analysis. To determine the association between urine protein concentration and pH or USG, Spearman rank correlation coefficients (r_{sp}) were calculated. The α value was set at 0.05, with inclusion of an experimental–wise correction to account for the number of measurands in the hemogram (n = 21, P = 0.0024) or biochemical profile (n = 0.0016). For the 2 correlations tested in urine, Bonferroni correction was used (P = 0.025).

Results

Blood samples were collected from 60 Southern pouched rats (36 males and 24 females). One animal had only a hemogram done, and another had only a biochemical panel done. No samples were excluded due to icterus, hemolysis or lipemia. Urine samples were obtained from 27 rats (11 males and 16 females). All results from 1 male rat were excluded because renal insufficiency was identified in the clinical pathologic test results, based on moderate increases in urea nitrogen (34 mmol/L) and creatinine (106 µmol/L) with concurrent isosthenuria (urine specific gravity, 1.013) and excessive proteinuria (1 g/L, 2+). This rat was found dead 1 mo after sample collection; histopathologic assessment of renal tissue on postmortem examination confirmed chronic kidney disease.

For the hematologic and biochemical analytes with identified outliers, reference intervals before outlier removal and details regarding the removed outliers are provided in Tables 4 and 5. Tables 6 and 7 provide reference intervals for analytes with and without outliers after removal of the outliers. The majority of variables followed a nonGaussian distribution. Platelet clumps were identified in smears of EDTA-anticoagulated blood from 30 animals. Excluding results from all rats with platelet clumps would leave too few animals to determine accurate reference intervals for the platelet count and MPV. Therefore, to increase the number of included animals, we reasoned that the automated platelet count was a minimum

Table 4. Original hematologic reference intervals (RI) in EDTA-anticoagulated blood samples from 59 healthy adult Southern giant pouched rats (*Cricetomys ansorgei*) before outlier removal. Results were obtained with the ADVIA 2120 hematology analyzer and a manual 100-cell differential cell count. Only variables for which outliers were removed from the final RIs (Table 6) are shown. The reason for each outlier removal is provided below the table. Note that 2 results for platelet count and MPV are provided; all 59 animals regardless of clumps and 29 animals in which no clumps were identified on blood smear examination. None of the data required transformation.

		Descriptive Statistics				RI with 90% CI				
Variable	Units	Mean	Median	SD	Min/Max	RI	Lower Limit	Upper Limit	*n*	Distribution
WBC	10^6/L	11.6	11.0	3.5	6.4–24.4	6.8–22.7	6.4–7.4	17.4–24.4	59	NG
SEG NEUT	10^6/L	2.2	1.7	2.1	0.2–14.2	0.4–11.0	0.2–0.8	5.0–14.2	59	NG
LYMPH	10^6/L	7.0	6.8	2.5	2.3–16	2.8–14.3	2.3–3.7	11.3–16	59	NG
MONO	10^6/L	1.0	0.9	0.6	0–3.7	0.1–3.2	0–0.3	1.8–3.7	59	NG
EOS	10^6/L	1.4	1.3	0.6	0.2–3.6	0.3–3.0	0.2–0.5	2.4–3.6	59	NG
Platelets with clumps	10^6/L	665	659	150	150–1071	261–1022	169–457	880–1071	59	NG
Platelets with no clumps	10^6/L	702	667	123	527–1071	nd*	nd	nd	29	G
MPV with clumps	fL	7.7	7.8	0.6	6.3–8.9	6.4–8.8	6.3–6.7	8.5–8.9	59	NG
MPV with no clumps	fL	7.6	7.7	0.5	6.5–8.6	6.5–8.8	6.3–8.9	8.5–9.0	29	G

Min/Max: Minimum/Maximum; SEG NEUT: Segmented neutrophils; LYMPH: Lymphocytes; MONO: Monocytes; EOS: Eosinophils; NG = Nongaussian; G = Gaussian.
* Interval invalid per program.
Outliers removed
WBC: Six outliers; 1) #9, WBC 24.4 × 10^6/L - outlier on program and on SEG NEUT count, 2) #36, WBC 21.0 × 10^6/L - outlier on program and on program with SEG NEUT and EOS counts, 3) #35, WBC 17.4 × 10^6/L - outlier on program with MONO count, 4) #46, WBC 18.9 × 10^6/L - outlier on program and on program with LYMPH count, 5) #53, 15.4 × 10^6/L - Not an outlier on program, but was an outlier on platelet count and had the highest percentage of granular lymphocytes. This animal developed a subcutaneous abscess 5 mo after the study was completed and could have had inflammation brewing at the time of the study; 6) #62, 16.8 × 10^6/L - This animal was a suspect outlier on the program. It had suffered a tail injury 7-8 wk prior to start of the study. Although the lesion appeared healed, there could have been a persistent inflammatory response.
SEG NEUT: Four outliers; 1) #9, SEG NEUT 14.2 × 10^6/L - outlier on program, 2) #36, SEG NEUT 7.8 × 10^6/L - outlier on program; 3) #53, 5.4 × 10^6/L – see above. This animal was also identified as an outlier by the program after removal of the other 3 highest values (from rats #9, #36 and #62); 4) #62, 5.7 × 10^6/L – outlier on program. See WBC above.
LYMPH: One outlier; #46, LYMPH 16.0 × 10^6/L - outlier on program.
MONO: Two outliers; 1) #35, MONO 3.7 × 10^6/L - outlier on program, 2) #53, MONO 2.6 × 10^6/L – possible outlier on program and outlier by visual inspection. This rat also had the highest granular lymphocyte percentage (26%) and a thrombocytosis.
EOS: One outlier; #36, EOS 3.6 × 10^6/L – possible outlier on program, outlier on program for WBC and outlier visually for EOS count.
Platelets: Eight removals; 1) Removed results of 7 animals with clumps and platelet counts below the lowest value for animals without clumps in the blood smear (527 × 10^6/L); 2) #53, 1071 × 10^6/L – suspect outlier on program. See above for WBC, SEG NEUT and MONO count.

Table 5. Original biochemical reference intervals (RI) in lithium-heparinized plasma from 59 healthy adult Southern giant pouched rats (*Cricetomys ansorgei*) before outlier removal. Results were obtained with the Hitachi P Modular (most analytes) and Cobas 501 (lipase) biochemical analyzers. Only analytes for which outliers were removed from the final RIs (Table 7) are shown. The reason for each outlier removal is provided below the table. None of the data required transformation.

		Descriptive statistics				RI with 90% CI				
Analyte	Units	Mean	Median	SD	Min/Max	RI	Lower Limit	Upper Limit	*n*	Distribution
Potassium	mmol/L	4.9	4.8	0.5	4.0–6.5	4.1–6.2	4.0–4.2	5.6–6.5	59	NG
Sodium: Potassium		30	30	3	22–36	23–35	22–25	34–36	59	NG
Bicarbonate	mmol/L	31	31	3	20–37	23–37	20–27	35–37	59	NG
Anion gap	mmol/L	19	19	4	9–38	12–32	9–15	23–38	59	NG
CK	IU/L	361	331	115	228–877	232–767	228–238	544–877	59	NG
Triglycerides	mmol/L	1.2	1.0	0.6	0.5–4.2	0.5–3.5	0.5–0.7	1.9–4.2	59	NG

Min/Max: Minimum/Maximum; CK: Creatine kinase; NG = Nongaussian
Outliers removed
Potassium and Sodium:Potassium: One outlier; #53, K 6.5 mmol/L – Possible outlier on program and visually.
Bicarbonate and anion gap: One outlier; #23, bicarbonate 20 mmol/L; anion gap 38 mmol/L – Outlier on program and visually.
CK: One outlier; #65, CK 877 IU/L – Outlier on program and visually.
Triglycerides: One outlier; #38, triglycerides 4.2 mmol/L – Outlier on the program and visually.

value in animals with observed clumps. We then included platelet counts from animals with clumps and an automated count above the minimum for animals without clumps (527 × 10^6/L). Similarly, we reasoned that platelet clumps would falsely increase the MPV and included results from animals with clumps and an MPV below the maximum for animals without clumps (84 g/L). These adjustments increased the number of animals for both analytes and allowed determination of reference intervals for platelet count and MPV (Table 6).

Neutrophils ranged from 11 to 13 µm in diameter and had irregular nuclear outlines and light pink granules in a colorless cytoplasm. Most neutrophils lacked distinct segmentation; a few had 2 to 7 nuclear segments with rare ring nuclei. Barr bodies were not identified. Eosinophils ranged from 11 to 15 µm in diameter and had irregular nuclear outlines, with

Table 6. Hematologic reference intervals (RI) for 59 clinically healthy adult Southern giant pouched rats (*Cricetomys ansorgei*)

		Descriptive statistics[a]				RI with 90% CI			
	Unit	Mean	Median	SD	Range	RI	Lower limit	Upper limit	Distribution
Hct	L/L	0.46	0.46	0.03	0.40 to 0.57	0.41 to 0.56	0.40 to 0.42	0.50 to 0.57	NG
Hgb	g/L	154	155	10	135 to 190	135 to 182	135 to 138	168 to 190	NG
RBC	10^9/L	6.4	6.4	0.5	5.2 to 8.0	5.4 to 7.8	5.2 to 5.7	7.2 to 8.0	NG
MCV	fL	72	72	3	66 to 79	67 to 79	66 to 68	77 to 79	NG
						66 to 78	65 to 68	77 to 79	G
MCH	pg	24	24	1	22 to 26	22 to 26	22 to 23	26 to 26	NG
MCHC	g/L	330	330	10	320 to 350	320 to 350	320 to 320	350 to 350	NG
RBC distribution width	%	12.9	13.1	0.9	11 to 14.5	11.1 to 14.4	11 to 11.4	14.2 to 14.5	NG
						11.2 to 14.7	10.9 to 11.5	14.4 to 15.0	G
Nucleated RBC	/100 WBC	0	0	0	0	0	—	—	—
WBC	10^6/L	10.8	11.0	2.4	6.4 to 16.7	6.6 to 16.5	6.4 to 7.3	14.8 to 16.7	NG
						5.9 to 15.7	5.0 to 6.8	14.7 to 16.6	G
Segmented neutrophils	10^6/L	1.8	1.6	0.9	0.2 to 5.3	0.3 to 4.9	0.2 to 0.7	2.9 to 5.3	NG
Band neutrophils	10^6/L	0	0	0.02	0.0 to 0.1	0.0 to 0.1	0	0 to 0.1	NG
Lymphocytes	10^6/L	6.9	6.8	2.2	2.3 to 12.5	2.7 to 12.1	2.3 to 3.7	10.2 to 12.5	NG
						2.8 to 11.9	2.2 to 3.5	10.9 to 12.7	Trans-G
Monocytes	10^6/L	0.9	0.9	0.4	0 to 1.8	0.1 to 1.8	0 to 0.3	1.7 to 1.8	NG
						0 to 1.8	–0.1 to 0.2	1.6 to 2.0	G
Eosinophils	10^6/L	1.4	1.3	0.6	0.2 to 2.4	0.3 to 2.4	0.2 to 0.5	2.3 to 2.4	NG
						0.2 to 2.5	0.1 to 0.4	2.3 to 2.7	G
Basophils	10^6/L	0.01	0	0.03	0 to 0.1	0 to 0.1	0	0.1 to 0.1	NG
Platelets	10^6/L	692	667	107	527 to 974	527 to 950	527 to 543	866 to 974	NG
						475 to 908	436 to 516	865 to 950	G
MPV	fL	7.7	7.8	0.6	6.3 to 8.6	6.4 to 8.6	6.3 to 6.6	8.4 to 8.6	NG
						6.2 to 8.6	5.8 to 6.6	8.5 to 8.8	Trans-G
Reticulocytes	%	1.3	1.1	0.7	0.3 to 4.0	0.4 to 3.4	0.3 to 0.5	2.5 to 4.0	NG
Absolute reticulocytes	10^6/L	82.8	73.5	42.7	18.9 to 229.8	23.7 to 198.9	18.9 to 30.2	159.0 to 229.9	NG
						22.4 to 190.3	17.5 to 28.0	164.9 to 219.2	Trans-G
Total protein	g/L	78	76	6	65 to 94	67 to 92	65 to 71	87 to 94	NG

–, Results were zero, so no intervals could be computed; G, Gaussian; NG, nonGaussian; Trans-G, Gaussian after Box–Cox transformation
Data were obtained from EDTA-anticoagulated by using an automated hematology analyzer and a manual 100-cell differential cell count, with plasma total protein measured by refractometry. Intervals were established after removal of outliers.
[a]Data were nontransformed.

rare cells having 2 nuclear segments. They contained numerous round light-pink granules in a light-blue cytoplasm. Basophils had nuclei of uniform width without segmentation and many round to elongate dull-red cytoplasmic granules (Figure 1). Lymphocytes predominated in blood, with a median neutrophil:lymphocyte ratio of 0.26 and range of 0.02 to 1.11 (outliers excluded). Lymphocytes were typically small (7 to 11 µm), with scant light to medium blue cytoplasm and round to oval to rarely lobulated nuclei with clumped chromatin. A few intermediate (9 to 13 µm) and large (13 to 16 µm) lymphocytes had clumped chromatin (Figures 1 and 2). Several lymphocytes had more cytoplasm (Figure 2). Several lymphocytes had reactive features, such as dark blue cytoplasm, or low numbers of clear discrete-margined cytoplasmic vacuoles (Figures 1 and 2). Granular lymphocytes comprised a median of 4% (range, 0% to 26%) of the lymphocytes (Figures 1 and 2). Median granular lymphocyte percentages did not differ between male (3.9%) and female (4.0%) rats ($P = 0.525$). Monocytes were the largest cells, ranging from 12 to 18 µm in size, and had round to oval nuclei with lightly clumped chromatin and light blue cytoplasm that usually contained low numbers of clear discrete-margined vacuoles (Figure 1). RBC were 5 to 6 µm in diameter and had a small central pallor. A few stomatocytes and polychromatophilic RBC were seen, and a single nucleated RBC was seen in one rat. Platelets were small (1 to 2.5 µm), with gray cytoplasm containing central red cytoplasmic granules (Figure 1).

After the exclusion of outliers, sex-dependent differences were found in the RBC count and RBC indices (Table 8). For the biochemical analytes, lower urea nitrogen concentrations and GGT activities were identified in male rats (Table 9).

Neutrophils were positive for all cytochemical stains, whereas monocytes were positive for ANBE and CAE, variably positive for ALP, and negative for myeloperoxidase. Eosinophil granules were positive for CAE and myeloperoxidase. Lymphocytes were negative with all stains, except for cells suspected to be granular lymphocytes, which had weak granular staining reactions for ALP and ANBE (Table 10, Figure 3). Too few basophils were seen to ascertain their cytochemical staining characteristics.

Urine ranged from light to dark yellow and was clear on visual inspection in most animals. Urine pH varied from 8 to greater than 9.0, with a mean urine specific gravity of 1.031 ± 0.011 (range, 1.011 to 1.048). The median total protein concentration was 0.2 g/dL (1+; range, negative to 1.0 g/L or 2+). Neither median pH (both sexes: 8.5, $P = 0.071$), urine specific gravity (male, 1.027 ± 0.010; female: 1.034 ± 0.010, $P = 0.125$), nor total

Table 7. Biochemical reference intervals (RI) for lithium-heparinized plasma from 59 clinically healthy adult Southern giant pouched rats (*Cricetomys ansorgei*)

		Descriptive statistics[a]				RI with 90% CI			
Analyte	Unit	Mean	Median	SD	Range	RI	Lower Limit	Upper Limit	Distribution
Sodium	mmol/L	144	143	3	137 to 150	138 to 150	137 to 140	149 to 150	NG
Potassium	mmol/L	4.9	4.8	0.4	4.0 to 5.9	4.1 to 5.8	4.0 to 4.2	5.6 to 5.9	NG
						4.1 to 5.8	3.9 to 4.2	5.6 to 6.0	Trans-G
Sodium:potassium		30	30	3	24 to 36	25 to 36	24 to 26	34 to 36	NG
Chloride	mmol/L	98	98	2	92 to 104	93 to 104	92 to 95	102 to 104	NG
Bicarbonate	mmol/L	31	31	2	27 to 37	27 to 37	27 to 28	34 to 37	NG
Anion gap	mmol/L	19	19	3	9 to 26	11 to 25	9 to 15	23 to 26	NG
Urea nitrogen	mmol/L	7.5	7.5	1.8	3.6 to 11.8	4.3 to 11.4	3.6 to 5.0	10.0 to 11.8	NG
						4.3 to 11.1	3.6 to 5.0	10.7 to 11.8	G
Creatinine	umol/L	35.4	35.4	8.8	17.7 to 61.9	17.7 to 53.0	17.7 to 26.5	44.2 to 61.9	NG
Calcium	mmol/L	2.7	2.7	0.1	2.6 to 2.9	2.6 to 2.9	2.6 to 2.6	2.9 to 2.9	NG
						2.6 to 2.9	2.6 to 2.6	2.9 to 2.9	G
Phosphate	mmol/L	1.2	1.2	0.3	0.7 to 1.9	0.8 to 1.9	0.7 to 0.8	1.7 to 1.9	NG
Magnesium	mmol/L	0.6	0.6	0.1	0.5 to 0.7	0.5 to 0.7	0.5 to 0.5	0.7 to 0.7	NG
Total protein	g/L	72	71	6	62 to 89	62 to 87	62 to 64	83 to 90	NG
						62 to 87	61 to 64	83 to 92	Trans-G
Albumin	g/L	28	29	3	22 to 34	22 to 34	22 to 24	33 to 34	NG
						22 to 34	22 to 24	33 to 35	G
Globulins	g/L	44	40	8	31 to 65	32 to 64	31 to 35	58 to 65	NG
Albumin:globulins		0.7	0.7	0.2	0.4 to 1.0	0.4 to 1.0	0.4 to 0.4	0.9 to 1.0	NG
Glucose	mmol/L	8.3	8.1	1.7	6.1 to 11.7	5.7 to 11.7	5.6 to 5.9	11.4 to 11.7	NG
						5.5 to 12.2	5.2 to 5.9	11.2 to 13.2	Trans-G
ALT	IU/L	21	19	10	11 to 68	12 to 58	11 to 13	38 to 68	NG
AST	IU/L	24	22	9	11 to 53	12 to 52	11 to 15	40 to 53	NG
GGT	IU/L	2	1	1	1 to 5	1 to 5	1 to 1	4 to 5	NG
ALP	IU/L	94	91	30	48 to 171	49 to 164	48 to 58	142 to 171	NG
Total bilirubin	umol/L	0.7	0	1.7	0 to 13.7	0 to 13.7	0 to 0	1.7 to 13.7	NG
Direct bilirubin	umol/L	0.2	0	0.5	0 to 1.7	0 to 1.7	0 to 0	1.7 to 1.7	NG
Indirect bilirubin	umol/L	0.5	0	1.7	0 to 13.7	0 to 13.7	0 to 0	1.7 to 13.7	NG
Creatine kinase	IU/L	352	331	93	228 to 657	231 to 607	228 to 238	518 to 657	NG
Iron	umol/L	31.9	31.3	9.1	14.1 to 64.3	15.0 to 57.6	14.1 to 20.6	47.6 to 64.3	NG
Total iron-binding capacity	umol/L	68.7	68.0	10.4	48.0 to 93.6	48.0 to 89.5	44.2 to 51.9	85.7 to 93.4	G
						49.8 to 90.9	46.0 to 52.8	86.3 to 95.2	Trans-G
Saturation	%	46	44	11	22 to 84	24 to 78	22 to 33	64 to 84	NG
Amylase	IU/L	786	770	148	458 to 1097	512 to 1096	458 to 585	1023 to 1097	NG
						488 to 1084	439 to 547	1028 to 1142	G
Lipase	IU/L	12	11	3	7 to 22	8 to 25	7 to 8	17 to 27	NG
						8 to 21	7 to 8	18 to 25	Trans-G
Cholesterol	mmol/L	3.2	3.1	0.8	1.8 to 5.5	1.8 to 5.3	1.8 to 2.1	4.7 to 5.5	NG
						1.9 to 5.1	1.8 to 2.1	4.7 to 5.6	Trans-G
Triglycerides	mmol/L	1.1	1.0	0.4	0.5 to 2.7	0.5 to 2.4	0.5 to 0.6	1.8 to 2.7	NG
Nonesterified fatty acids	mmol/L	0.26	0.23	0.09	0.11 to 0.49	0.12 to 0.48	0.11 to 0.14	0.43 to 0.49	NG
						0.12 to 0.47	0.10 to 0.14	0.42 to 0.53	Trans-G

G, Gaussian; NG, nonGaussian; Trans-G, Gaussian after Box–Cox transformation.
Data were obtained by using automated biochemical analyzers.
[a]Data were nontransformed.

protein concentration (both sexes: 0.3 g/L, $P = 0.467$) differed between male and female animals. Urine protein correlated with urine specific gravity ($r_{sp} = 0.667$, $P < 0.001$) but not pH ($r_{sp} = –0.09$, $P = 0.656$, Figure 4). Four animals had variable reactions for blood on the dipstick; 3 of these animals had fewer than 5 RBC/HPF, and the remaining one had mild hematuria (20 to 100 RBC/HPF). One male animal had trace glucosuria with a urine specific gravity of 1.041 and a blood glucose concentration of 125 mg/dL. This animal remained clinically healthy for 2 y after completion of this study. Twelve animals had trace to slight ketones (their weak positive ketone results on the dipstick were not verified with reagent tablets). Eight animals had a slight bilirubin reaction (2 males and 6 females), all 8 had a urine specific gravity of 1.028 or greater; these positive bili-

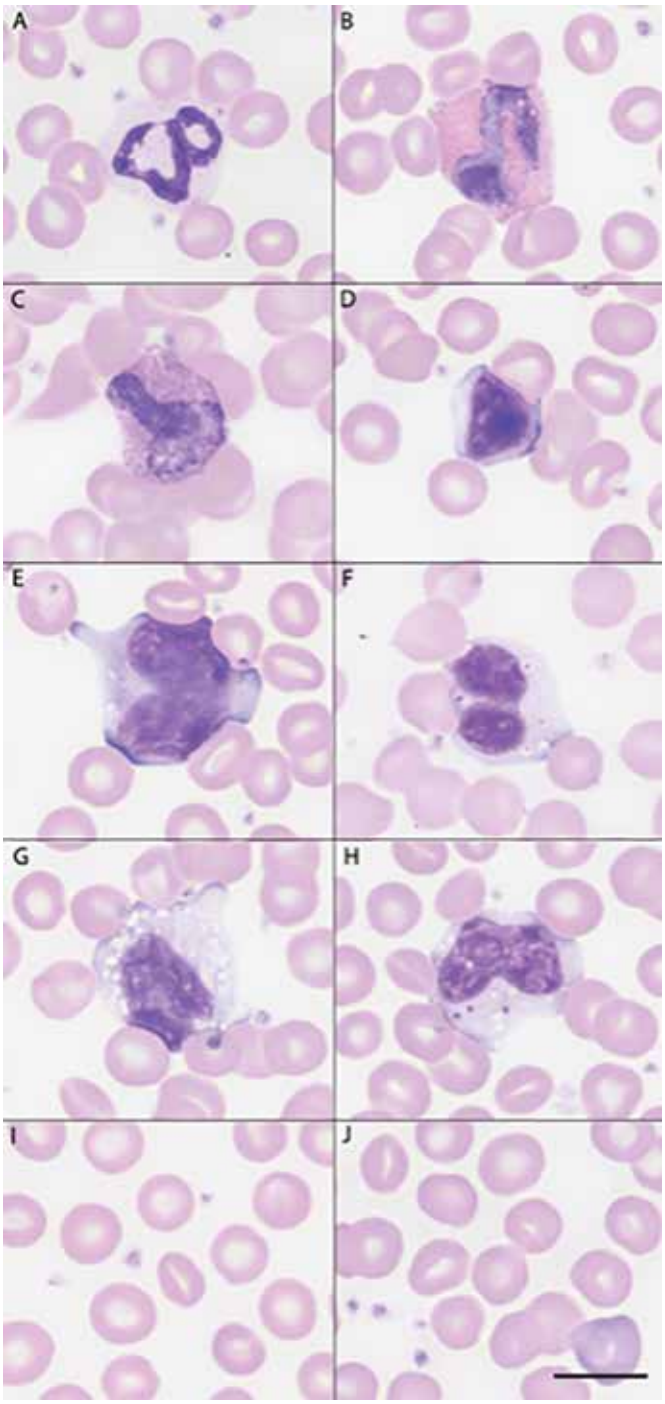

Figure 1. Compilation of representative cropped images of peripheral blood cells from wild-caught Southern giant pouched rats (*Cricetomys ansorgei*) stained with a modified Wright's stain. As for other species, granulocytes could be distinguished by the color of the cytoplasmic granules. (A) Neutrophil. (B) Eosinophil. (C) Basophil. Most of the (D) lymphocytes were small cells, with low numbers of (E) reactive and (F) granular forms; granular lymphocytes had increased amounts of light blue cytoplasm. (G and H) Monocytes usually contained cytoplasmic vacuoles, with rare cells having less than 5 small round red (not shown) or (H) blue granules. (I and J) RBC had small amounts of central pallor, with low numbers of (I and J) stomatocytic and (J) polychromatophilic RBC. (I and J) Platelets were small cells with red cytoplasmic granules. Scale bar, 10 µm.

rubin dipstick results were confirmed by using reagent tablets in the 6 females but not the 2 males (reagent tablet yielded a negative result). All animals had fewer than 5 WBC/HPF. In a few animals, the urine sediment had a few epithelial cells, fat droplets, or calcium oxalate dihydrate crystals, but none had casts, bacteria, or sperm.

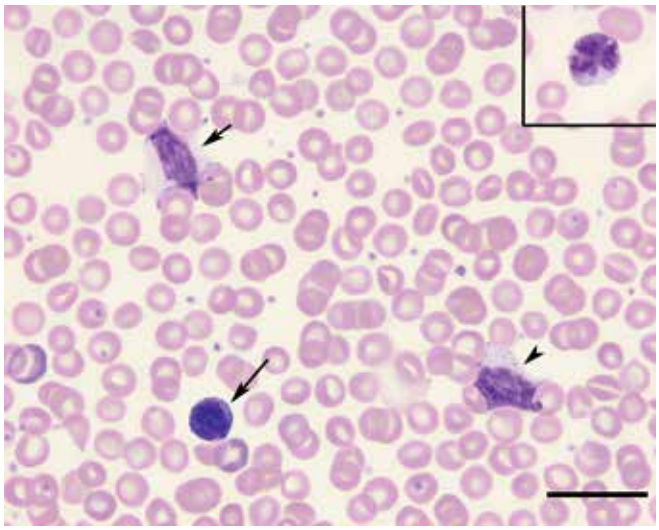

Figure 2. Representative images of various types of lymphocytes seen in modified Wright–stained blood smears from Southern giant pouched rats (*Cricetomys ansorgei*). These cells included low numbers of small (long arrow) and large (not shown) reactive lymphocytes; the cytoplasm of reactive lymphocytes was bluer than typical. Small (not shown) to intermediate (short arrow) lymphocytes had increased amounts of light-blue cytoplasm, and granular lymphocytes (arrowhead) were seen also. The inset was cropped from a different animal and shows a lymphocyte with a lobulated nucleus at the same magnification as the rest of the image; these lymphocytes were seen only rarely. Scale bar, 20 µm.

Discussion

Here we present data for hematologic and biochemical analytes for adult wild-caught SGP rats, along with the cytochemical staining reactions of peripheral blood leukocytes and urinalysis findings. Our results will provide a useful resource for investigators or veterinarians working with these animals and will help to confirm their health or identify underlying disease.

Working with wild-caught animals presents several limitations. Age can only be estimated, pregnancy may not be detected unless advanced, and animals may be harboring infectious organisms or suffering from diseases that are not clinically evident. Handling for blood sample collection or anesthesia may induce a corticosteroid or sympathetic nervous response in animals not acclimated to these procedures.[10] These uncontrollable variables affect clinical pathologic data and potentially broaden reference intervals, which thus might be less sensitive to disease-associated changes.

Our data showed substantial variability in total leukocyte, neutrophil, and lymphocyte counts, even after the removal of statistical or visual outliers. We suspect that a catecholamine response, which would result in a mature neutrophilia and lymphocytosis,[2,17] contributed to this variability. Wild-caught dusky-footed woodrats (*Neotoma fuscipes*) and Australian bush rats (*Rattus fuscipes*) had similarly wide intervals for neutrophil and lymphocyte concentrations,[22,42] whereas laboratory-bred Sprague–Dawley rats have tighter intervals.[14] A catecholamine response also would explain the high glucose concentrations in our SGP rats and other studies of wild-caught dusky-footed wood rats or Australian Bush rats[22,42] but not 9-wk-old laboratory-bred Sprague–Dawley rats.[14] A stress response due to endogenous corticosteroids cannot be ruled out; however none of the SGP rats had a neutrophilia with a

Table 8. Hematologic variables partitioned by sex for EDTA-anticoagulated blood samples from 59 wild-caught Southern giant pouched rats (*Cricetomys ansorgei*)

		Male (n = 29–35)			Female (n = 21–24)		
	Unit	Mean ± 1 SD	Median	Range	Mean ± 1 SD	Median	Range
Hct	L/L	0.47 ± 0.03	0.47	0.41 to 0.47	0.45 ± 0.02	0.46	0.40 to 0.50
Hgb	g/L	157 ± 10	159	135 to 190	149 ± 09	149	135 to 167
RBC	10^9/L	6.6 ± 0.5[a]	6.8	5.6 to 8.0	6.1 ± 0.3	6.2	5.2 to 6.8
MCV	fL	77 ± 3[a]	77	66 to 78	74 ± 3	74	68 to 79
MCH	pg	24 ± 1	24	22 to 26	25 ± 1	25	22 to 26
MCHC	g/L	340 ± 10	340[a]	320 to 350	330 ± 10	330	320 to 340
RBC distribution width	%	13.4 ± 0.6[a]	13.3	12.1 to 14.5	12.3 ± 0.8	12.1	11 to 14.1
Nucleated RBC	/100 WBC	0	0	0 to 0	0	0	0 to 0
WBC	10^6/L	11.0 ± 2.6	11.1	6.4 to 16.7	10.5 ± 2.1	10.5	6.8 to 14.8
Segmented neutrophils	10^6/L	1.8 ± 0.9	1.5	0.2 to 5.3	1.7 ± 0.8	1.6	0.5 to 4.2
Band neutrophils	10^6/L	0 ± 0.02	0	0 to 0.1	0 ± 0.02	0	0 to 0.1
Lymphocytes	10^6/L	7.1 ± 2.4	7.3	3.2 to 12.5	6.5 ± 1.9	6.8	2.3 to 10.1
Monocytes	10^6/L	1.0 ± 0.4	1.0	0 to 1.8	0.8 ± 0.4	0.7	0.1 to 1.8
Eosinophils	10^6/L	1.3 ± 0.5	1.3	0.3 to 2.4	1.5 ± 0.6	1.6	0.2 to 2.4
Basophils	10^6/L	0 ± 0.03	0	0 to 0.1	0 ± 0.02	0	0 to 0.1
Platelets	10^6/L	685 ± 116	659	527 to 974	700 ± 96	699	535 to 832
MPV	fL	7.5 ± 0.6	7.5	6.3 to 8.5	7.8 ± 0.5	7.9	6.8 to 8.6
Reticulocytes	%	1.5 ± 0.7	1.3	0.4 to 4.0	1.0 ± 0.6	0.8	0.3 to 2.8
Absolute reticulocytes	10^6/L	97.4 ± 41.5[a]	91.8	28.5 to 229.9	61.4 ± 35.3	48.6	18.9 ± 167.9
Total protein	g/L	77 ± 6	76	65 to 94	78 ± 6	77	68 to 87

Data were obtained by using an automated hematology analyzer and a manual 100-cell differential cell count, with plasma total protein measured by refractometry. Outliers identified during reference interval determination were excluded from this analysis, resulting in varying animal numbers.
[a]Means (Gaussian data) and medians (nonGaussian data) were significantly different from those for female rats after the application of experiment-wise correction ($P < 0.0024$) for the number of measurands (n = 21).

concurrent lymphopenia—hematologic findings compatible with a stress leukogram.[37] A stress response was suspected, in light of high neutrophil:lymphocyte ratios, in dusky-footed woodrats that were sampled after capture as compared with those housed in the laboratory for 1 to 6 d after capture.[42] In general, lymphocytes predominate in the blood of wild-caught or laboratory-raised rats, and very low neutrophil counts (<1 × 10^6/L) can be normal for the order Rodentia.[14,22,42] Perhaps as our SGP rats become accustomed to handling, narrower reference intervals for the relevant leukocytes and lower intervals for glucose concentration would be obtained on repeat testing. However, due to financial constraints and ethical considerations (e.g., avoidance of additional anesthesia), clinical pathologic testing was not repeated on these animals.

We retained most of the outliers that were identified by the statistical program as 'definitive' or 'suspect.' These outliers could represent animals with underlying disease. For example, creatinine concentrations were quite low in our SGP rats and in other species of wild-caught or laboratory-bred rats.[14,22,28,42] Our SGP rats had higher globulin concentrations than other species of wild-caught or laboratory-bred rats,[14,22,42] suggesting that they might have underlying subclinical disease, inducing antigenic stimulation. However, the high globulin concentrations might reflect true differences between species or be related to the instrumentation or testing methods used in the various studies. Although granular lymphocytes were seen in several blood smears from our SGP rats, the animal with the highest percentage of granular lymphocytes (26%) had a concurrent monocytosis and thrombocytosis, suggesting that the high percentage of granular lymphocytes was due to underlying subclinical disease; this rat was excluded as an outlier for both monocytes and platelets. An underlying clinical condition was not confirmed by additional diagnostic testing in this animal, but it did present with a subcutaneous abscess 5 mo later, which could have been developing at the time of sample collection for the study. In addition, our SGP rats had higher eosinophil counts than previously reported for other species of wild-caught or laboratory-bred rats.[14,22,42] The higher counts could be explained by residual tissue inflammation from endo- or ectoparasitism, such as the gastrointestinal nematodes and earwigs detected in our SGP rats on their arrival to the United States, despite apparent successful treatment prior to the start of this study. Gastrointestinal nematodes and coccidia have been previously identified on fecal analysis from SGP rats.[4]

Other preanalytical variables that influence results of clinical pathologic testing are the type of anesthetic agent used for immobilization, the sample collection site, and timing of blood collection in relation to feeding and time of day.[10,23,41] We used isoflurane and arterial blood from the tail for anesthesia and blood sampling, respectively, in this study. Minor changes are seen in hematologic and biochemical test results in Crl:WI BR, Crl:CD (SD) and F344/DuCrlCrlJ laboratory-bred rats anesthetized with isoflurane compared with other inhalant or injectable anesthetics,[5,25,38] and isoflurane is a recommended anesthetic for use in blood collection.[5] Few published reports on clinical pathologic testing compare arterial and venous blood samples.[3] Penetration of perivascular muscle during blood sample collection or excess handling of the animal[46] could have contributed to the wide range of creatine kinase activities we found in our study, although our results for creatine kinase activity in SGP rats were lower than those reported for wild-caught Australian bush rats in which samples were collected from the subclavian vein.[22] The SGP rats in our current study were not fasted before

Table 9. Biochemical variables partitioned by sex for lithium-heparinized plasma samples from 59 wild-caught Southern giant pouched rats (*Cricetomys ansorgei*)

		Male (n = 24–35)			Female (n = 23 or 24)		
Variable	Unit	Mean ± 1 SD	Median	Range	Mean ± 1 SD	Median	Range
Sodium	mmol/L	143 ± 3	142	137 to 149	144 ± 2	144	141 to 150
Potassium	mmol/L	4.9 ± 0.4	4.8	4.1 to 5.9	4.7 ± 0.4	4.6	4.0 to 5.6
Sodium:potassium		29 ± 3	30	24 to 34	31 ± 3	31	25 to 36
Chloride	mmol/L	98 ± 3	98	92 to 104	99 ± 2	99	95 to 101
Bicarbonate	mmol/L	31 ± 2	31	27 to 34	32 ± 2	32	29 to 37
Anion gap	mmol/L	20 ± 3	20	14 to 28	18 ± 3	19	9 to 23
Urea nitrogen	mmol/L	6.8 ± 1.4	6.8[a]	4.6 to 9.6	8.9 ± 1.4	9.3	3.6 to 11.8
Creatinine	umol/L	35.4 ± 8.8	35.4	17.7 to 79.6	35.4 ± 8.8	35.4	26.5 to 44.2
Calcium	mmol/L	2.7 ± 0.1	2.7	2.6 to 2.9	2.8 ± 0.1	2.8	2.6 to 2.9
Phosphate	mmol/L	1.2 ± 0.2	1.2	0.7 to 1.9	1.2 ± 0.3	1.2	0.8 to 1.9
Magnesium	mmol/L	0.6 ± 0.1	0.6	0.5 to 0.7	0.6 ± 0.1	0.6	0.5 to 0.7
Total protein	g/L	72 ± 6	71	64 to 89	73 ± 6	72	62 to 85
Albumin	g/L	28 ± 3	29	24 to 33	29 ± 3	29	22 to 34
Globulins	g/L	44 ± 08	40	35 to 65	44 ± 9	42	31 to 62
Albumin:globulins		0.7 ± 0.2	0.7	0.4 to 0.9	0.7 ± 0.2	0.7	0.4 to 1.0
Glucose	mmol/L	8.4 ± 1.8	8.0	5.6 to 11.7	8.1 ± 1.5	7.8	5.8 to 11.0
ALT	IU/L	22 ± 11	19	11 to 68	20 ± 6	19	13 to 41
AST	IU/L	22 ± 9	21	11 to 53	25 ± 9	22	15 to 30
ALP	IU/L	97 ± 32	97	48 to 171	90 ± 25	80	57 to 142
GGT	IU/L	1 ± 1	1[a]	1 to 5	2 ± 1	3	1 to 4
Total bilirubin	µmol/L	0.3 ± 0.7	0	0 to 1.7	1.7 ± 1.7	0.1	0 to 13.7
Direct bilirubin	µmol/L	0	0	0	0.3 ± 0.7	0	0 to 1.7
Indirect bilirubin	µmol/L	0.3 ± 0.7	0	0 to 1.7	1.7 ± 1.7	0	0 to 1.7
Creatine kinase	IU/L	327 ± 79	322	228 to 657	390 ± 101	390	235 to 552
Iron	µmol/L	30.3 ± 8.4	30.8	14.1 to 50.8	34.2 ± 11.3	33.3	22.4 to 64.3
Total iron-binding capacity	µmol/L	65.7 ± 10.2	66.4	48.0 to 84.5	73.2 ± 9.0	69.8	62.3 to 93.6
Saturation	%	46 ± 10	46	22 to 67	47 ± 12	43	33 to 84
Amylase	IU/L	783 ± 152	760	458 to 1097	791 ± 144	789	569 to 1058
Lipase	IU/L	11 ± 2	11	7 to 16	13 ± 4	12	9 to 27
Cholesterol	mmol/L	3.2 ± 0.9	2.9	1.8 to 5.5	3.2 ± 0.6	3.2	1.8 to 4.7
Triglycerides	mmol/L	1.2 ± 0.4	1.0	0.5 to 2.7	1.1 ± 0.3	1.0	0.5 to 2.1
Nonesterified fatty acids	mmol/L	0.26 ± 0.07	0.25	0.13 to 0.43	0.26 ± 0.11	0.22	0.11 to 0.49

Data were obtained by using biochemical analyzers. Outliers identified during reference interval determination were excluded from this analysis, resulting in the differing animal numbers.

[a]Medians were significantly different from those for female rats after the application of experimental-wise correction ($P < 0.0016$) for the number of measurands (n = 32).

Table 10. Cytochemical staining reactions in peripheral blood leukocytes from wild-caught Southern giant pouched rats

Cell type	Alkaline phosphatase	α-Naphthyl butyrate esterase	Chloroacetate esterase	Myeloperoxidase
Neutrophil	3+ (G)	1+ (C, multifocal)	3+ (G)	2+ (G)
Lymphocyte	Neg	Neg	Neg	Neg
Granular lymphocyte	1+ (G)	1+ (G)[a]	inconclusive	inconclusive
Monocyte	±; 1+ (G)	2-3+ (C, diffuse)	2+ (C, diffuse)	Neg
Eosinophil	negative	negative	1+ (G)	3+ (G)

Staining was performed in 2 male and 2 female rats; unless otherwise noted, results were consistent among animals. Lineages with cells displaying positive or negative staining reactions were designated as ±, with staining in positive cells subjectively graded by a single observer (TS) as weak (1+), moderate (2+), or strong (3+). Staining localization was noted within granules (G) or the cytoplasm (C) of each cell type, with the pattern of cytoplasmic staining further distinguished as focal, multifocal, or diffuse. Insufficient basophils were seen to determine their cytochemical staining reactions. Inconclusive indicates that the cell in question was not conclusively identified with the stain.

[a]ANBE-positive granular lymphocytes were only identified in the 2 female rats.

testing and had higher triglyceride concentrations than fasted Sprague–Dawley rats[14] but similar triglyceride concentrations to nonfasted wild-caught Australian bush rats[22] or 8 nonfasted captive adult Gambian rats.[28] Cholesterol concentrations were higher in SGP rats and in other species of wild-caught rats[22,28,42] as compared with laboratory-bred Sprague–Dawley rats;[14] this difference may be related to the timing of collection in relation to feeding or may be true species differences.

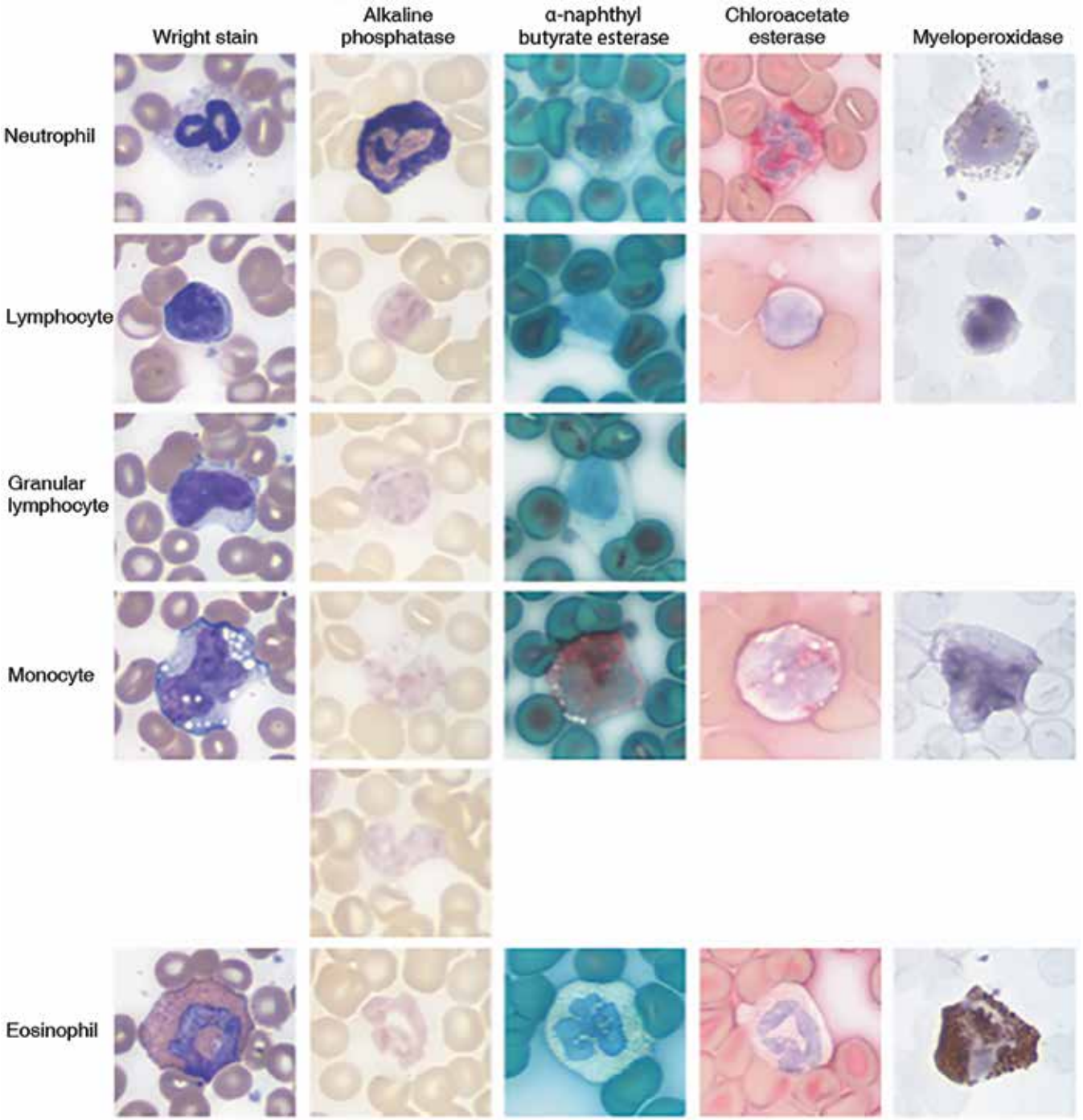

Figure 3. Compilation of representative cropped images of peripheral blood leukocytes of wild-caught Southern giant pouched rats (*Cricetomys ansorgei*) stained with modified Wright stain and various cytochemical stains. Granular lymphocytes were not conclusively identified in chloroacetate- and myeloperoxidase-stained slides and were presumed negative for these stains. Monocytes showed variable reactivity with alkaline phosphatase; both positive (upper panel) and negative (lower panel) stained monocytes are shown. Refer to Table 10 for the intensity and localization of staining reactions.

Beyond differences attributable to analytical variation (using the highest observed total error of the controls), sex-related differences were identified for the RBC count and MCV. Laboratory-bred male Sprague–Dawley, CD or Sand (*Psammomus obesus*) rats similarly have higher RBC counts and Hgb concentrations than do females;[14,18,19,26,27] these differences can be explained by the influences of sex hormones on erythropoiesis.[6,21] RBC counts, Hgb concentration, and Hct all decrease in laboratory-bred male Sprague–Dawley or CD rats after gonadectomy.[27,30] The higher MCV in the male SGP rats of our current study is similar to that reported for laboratory-bred Sprague–Dawley, CD or Sand rats[14,18,19,26] and may be attributable to the higher absolute reticulocyte counts in this study. Sex-associated differences in GGT activity were mild and considered clinically meaningless. The urea nitrogen concentration was significantly lower in males as compared with females in the current study but not in other studies in laboratory-bred sand rats[18] or pet rats.[15] The reason for this finding is unclear, but it might reflect sex- or species-related differences in anabolic or catabolic rates or glomerular filtration rate.

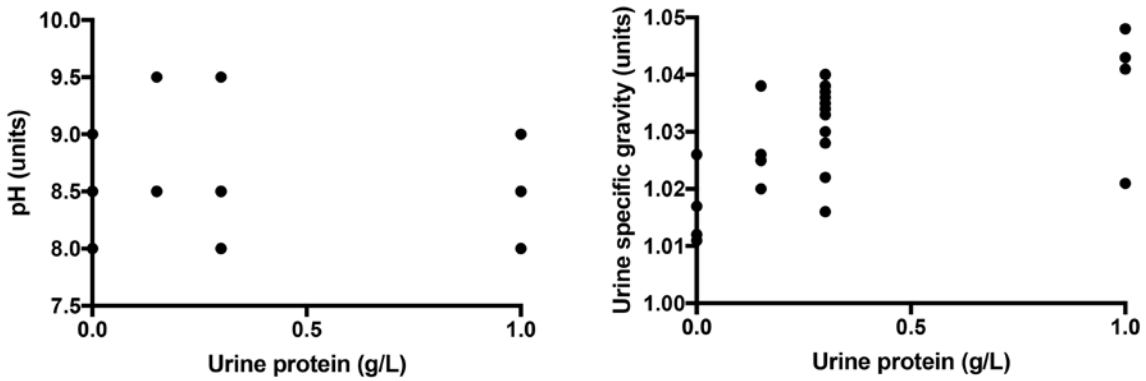

Figure 4. Correlation between pH (left panel, r_{sp} = −0.09, P = 0.656) or urine specific gravity (right panel, r_{sp} = 0.667, $P < 0.001$) and dipstick semiquantitative measurements of protein concentrations in urine samples from 27 Southern giant pouched rats (*Cricetomys ansorgei*). The urine analyzer did not provide a pH above 9.0; values reported as greater than 9.0 were designated as 9.5 for statistical purposes.

The cytochemical staining reactions that we obtained for ANBE and CAE in neutrophils, monocytes, and eosinophils in our SGP rats are similar to those reported for the other nonspecific esterase, α-naphthyl acetate esterase, and CAE in these leukocytes in Fischer rats (that study did not provide strain, substrain, or source),[29] with the exception of eosinophils, which were negative for CAE in Fischer rats but were weakly positive for CAE in the SGP rats of the current study. Granular staining reactions for myeloperoxidase were seen in neutrophils and eosinophils of our SGP rats, indicating it can be used as a marker for infiltrates of these cells in tissues, as in laboratory-bred rodents.[35] With the exception of granular forms, lymphocytes were negative for all cytochemical stains. Granular lymphocytes displayed weak positive cytoplasmic reactions for ALP and ANBE, as described for dogs.[44] The SGP rats in our study had positive reactions for ALP in neutrophils but not eosinophils, in contrast to Sprague–Dawley rats, in which neutrophils and eosinophils are positive for ALP.[45] Our results support species-specific reactions for cytochemical staining reactions.

A mild proteinuria may be normal in SGP rats, particularly in more concentrated urine. Urine protein concentrations of 0.13 to 5.0 g/L have been reported for Sprague–Dawley rats in which urine pH ranges from 6.4 to 8.2.[36] We did not measure urine protein concentrations or protein:creatinine ratios on the biochemistry analyzer to verify the positive dipstick reactions, and the dipstick method may be unreliable as a rapid assessment of urine protein concentrations.[7] We considered false-positive reactions due to alkaline urine unlikely, because the urine protein did not correlate with pH. In contrast, we found a moderate correlation between urine protein concentration and urine specific gravity. Positive reactions for blood on the dipstick were attributed to collection-associated hemorrhage. The trace glucosuria detected by dipstick for one rat might have been a false positive, because the result was not verified on a biochemical analyzer. Bilirubinuria can be normal in rat urine, due to conjugation of bilirubin in renal tubular epithelial cells.[9,33]

In conclusion, this study provides reference intervals for hematologic and biochemical analytes and urine findings in SGP rats. These intervals can be used as a guide to identify natural or experimentally-associated changes in individual or groups of rats. As an example, we recently used these reference intervals to detect inflammation, liver and pancreatic injury and hyperglycemia in a SGP rat that recovered slowly from anesthesia and had a slightly distended abdomen, with marked glucosuria. The animal was ultimately euthanized and steatohepatitis was detected on post-mortem examination. Similarly, the described morphologic features of blood cells and cytochemical staining reactions of leukocytes can help with identification of disease, including hematopoietic neoplasms. Future studies could include serial sampling of SGP rats to determine biologic variation in clinical pathologic analytes, assess whether intervals change in laboratory-bred rats, and detect subclinical diseases, such as diabetes mellitus. In addition, testing of commercially available antibodies for phenotyping leukocytes would be useful in the SGP rats to associate changes in leukocytes or leukocyte subsets (e.g., helper/regulatory to cytotoxic T cell ratios) with disease, environmental stress or experimental conditions.

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References

1. **American Association Laboratory Animal Science.** [Internet]. 2021. AALAS position papers. [Cited 16 August 2021]. Available at: https://www.aalas.org/about-aalas/position-papers/humane-care-and-use
2. **Benschop RJ, Rodriguez-Feuerhahn M, Schedlowski M.** 1996. Catecholamine-induced leukocytosis: early observations, current research, and future directions. Brain Behav Immun **10:**77–91. https://doi.org/10.1006/brbi.1996.0009.
3. **Chan YK, Davis PF, Poppitt SD, Sun X, Greenhill NS, Krishnamurthi R, Prezepiorski A, McGill A, Krissansen GW.** 2012. Influence of tail versus cardiac sampling on blood glucose and lipid profiles in mice. Lab Anim **46:**142–147. https://doi.org/10.1258/la.2011.011136.
4. **Cullin CO, Sellers MS, Rogers ER, Scott KE, Lee DN, Ophir AG, Jackson TA.** 2017. Intestinal parasites and anthelmintic treatments in a laboratory colony of wild-caught African pouched rats (*Cricetomys ansorgei*). Comp Med **67:**420–429.
5. **Deckardt K, Weber I, Kaspers U, Hellwig J, Tennekes H, van Ravenzwaay B.** 2007. The effects of inhalation anaesthetics on common clinical pathology parameters in laboratory rats. Food Chem Toxicol **45:**1709–1718. https://doi.org/10.1016/j.fct.2007.03.005.
6. **Delev DP, Davcheva DP, Kostadinov ID, Kostadinova II.** 2013. Effect of testosterone propionate on erythropoiesis after experimental orchiectomy. Folia Med (Plovdiv) **55:**51–57. https://doi.org/10.2478/folmed-2013-0017.

7. **Doss GA, Mans C, Houseright RA, Webb JL.** 2016. Urinalysis in chinchillas (*Chinchilla lanigera*). J Am Vet Med Assoc **248:**901–907. https://doi.org/10.2460/javma.248.8.901.
8. **eClinPath.com.** [Internet]. 2020. Interference indices. [Cited 30 November 2020]. Available at: https://eclinpath.com/chemistry/interference-indices/.
9. **Elías MM, Mottino AD, Ochoa EJ.** 1997. Transepithelial fate of bilirubin in the isolated rat kidney. Biochim Biophys Acta **1336:**7–14. https://doi.org/10.1016/S0304-4165(97)00003-2.
10. **Everds NE.** 2017. Deciphering sources of variability in clinical pathology-it's not just about the numbers. Toxicol Pathol **45:**275–280. https://doi.org/10.1177/0192623316680783.
11. **Friedrichs KR, Harr KE, Freeman KP, Sladovitz B, Walton RM, Barnhart KF, Blanco-Chavez J.** 2012. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Vet Clin Pathol **41:**441–453. https://doi.org/10.1111/vcp.12006.
12. **Geffré A, Braun JP, Trumel C, Concordet D.** 2009. Estimation of reference intervals from small samples: an example using canine plasma creatinine. Vet Clin Pathol **38:**477–484. https://doi.org/10.1111/j.1939-165X.2009.00155.x.
13. **Harr KE, Flatland B, Nabity M, Freeman KP.** 2013. ASVCP guidelines: allowable total error guidelines for biochemistry. Vet Clin Pathol **42:**424–436. https://doi.org/10.1111/vcp.12101. Correction. 2018. Vet Clin Pathol **47:**170–170.
14. **He Q, Su G, Liu K, Zhang F, Jiang Y, Gao J, Liu L, Jiang Z, Jin M, Xie H.** 2017. Sex-specific reference intervals of hematologic and biochemical analytes in Sprague–Dawley rats using the nonparametric rank percentile method. PLoS One **12:**1–18. https://doi.org/10.1371/journal.pone.0189837.
15. **Houtmeyers A, Duchateau L, Grunewald B, Hermans K.** 2016. Reference intervals for biochemical blood variables, packed cell volume, and body temperature in pet rats (*Rattus norvegicus*) using point-of-care testing. Vet Clin Pathol **45:**669–679. https://doi.org/10.1111/vcp.12419.
16. **Institute for Laboratory Animal Research**. 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
17. **Iversen PO, Stokland A, Rolstad B, Benestad HB.** 1994. Adrenaline-induced leucocytosis: recruitment of blood cells from rat spleen, bone marrow and lymphatics. Eur J Appl Physiol Occup Physiol **68:**219–227. https://doi.org/10.1007/BF00376770.
18. **Kane JD, Steinbach TJ, Sturdivant RX, Burks RE.** 2012. Sex-associated effects on hematologic and serum chemistry analytes in sand rats (*Psammomys obesus*). J Am Assoc Lab Anim Sci **51:**769–774.
19. **Kong W-N, Niu Q-M, Ge L, Zhang N, Yan S-F, Chen W-B, Chang Y-W, Zhao S-E.** 2014. Sex differences in iron status and hepcidin expression in rats. Biol Trace Elem Res **160:**258–267. https://doi.org/10.1007/s12011-014-0051-3.
20. **La Londe KB, Mahoney A, Edwards TL, Cox C, Weetjens B, Durgin A, Poling A.** 2015. Training pouched rats to find people. J Appl Behav Anal **48:**1–10. https://doi.org/10.1002/jaba.181.
21. **Malgor LA, Barrios L, Blanc CC.** 1975. Effects of testosterone on bone marrow erythroid cells of normal and nephrectomized rats. Acta Physiol Lat Am **25:**179–187.
22. **McDonogh TL, Thompson PP, Sangster CR, Meagher PJB, Vogelnest L.** 2015. Hematologic and Biochemical reference values of the Australian bush rat (*Rattus fuscipes*). J Wildl Dis **51:**795–799. https://doi.org/10.7589/2014-08-201.
23. **Miwa K, Tamai S, Kinpara Y, Komatsu S, Goto M, Iguchi T, Suzuki T, Takasaki W, Mori K.** 2015. Impact of different blood sampling techniques on plasma biomarkers for skeletal myopathy in conscious rats. Fundam Toxicol Sci **2:**25–36. https://doi.org/10.2131/fts.2.25.
24. **Nabity MB, Harr KE, Camus MS, Flatland B, Vap LM.** 2018. ASVCP guidelines: Allowable total error hematology. Vet Clin Pathol **47:**9–21. https://doi.org/10.1111/vcp.12583.
25. **Nakatsu N, Igarashi Y, Aoshi T, Hamaguchi I, Saito M, Mizukami T, Momose H, Ishii KJ, Yamada H.** 2017. Isoflurane is a suitable alternative to ether for anesthetizing rats prior to euthanasia for gene expression analysis. J Toxicol Sci **42:**491–497. https://doi.org/10.2131/jts.42.491.
26. **Nemeth N, Kiss F, Furka I, Miko I.** 2010. Gender differences of blood rheological parameters in laboratory animals. Clin Hemorheol Microcirc **45:**263–272. https://doi.org/10.3233/CH-2010-1303.
27. **Nemeth N, Kiss F, Magyar Z, Miszti-Blasius K, Furka I.** 2012. Following-up hemorheological consequences of gonadectomy in male and female rats. Clin Hemorheol Microcirc **50:**231–243. https://doi.org/10.3233/CH-2011-1430.
28. **Onwuka SK, Nssien MAS, Olayemi FO, Olusola A.** 2003. Further studies on the plasma biochemistry of the african giant rat (*Cricetomys gambianus, Waterhouse*). Afr J Biomed Res **6:**33–36.
29. **Osbaldiston GW, Sullivan RJ.** 1978. Cytochemical demonstration of esterases in peripheral blood leukocytes. Am J Vet Res **39:**683–685.
30. **Pequignot JM, Spielvogel H, Caceres E, Rodriguez A, Semporé B, Pequignot J, Favier R.** 1997. Influence of gender and endogenous sex steroids on catecholaminergic structures involved in physiological adaptation to hypoxia. Pflugers Arch **433:**580–586. https://doi.org/10.1007/s004240050317.
31. **Poling A, Mahoney A, Beyene N, Weetjens B, Cox C, Durgin A.** 2015. Using giant African pouched rats to detect human tuberculosis: a review. Pan Afr Med J **21:**333. https://doi.org/10.11604/pamj.2015.21.333.2977.
32. **Poling A, Weetjens BJ, Cox C, Beyene N, Bach H, Sully A.** 2015. Teaching giant African pouched rats to find landmines: operant conditioning with real consequences. Behav Anal Pract **3:**19–25. https://doi.org/10.1007/BF03391761.
33. **Robinson SH, Yannoni C, Nagasawa S.** 1971. Bilirubin excretion in rats with normal and impaired bilirubin conjugation: effect of phenobarbital. J Clin Invest **50:**2606–2613. https://doi.org/10.1172/JCI106761.
34. **San Diego Zoo.** [Internet]. 2020. African giant pouched rat (*Cricetomyces gambianus*). [Cited November 30 2020]. Available at: https://animals.sandiegozoo.org/animals/african-giant-pouched-rat
35. **Schneider T, van Velzen D, Moqbel R, Issekutz AC.** 1997. Kinetics and quantitation of eosinophil and neutrophil recruitment to allergic lung inflammation in a brown Norway rat model. Am J Respir Cell Mol Biol **17:**702–712. https://doi.org/10.1165/ajrcmb.17.6.2849.
36. **Shevock PN, Khan SR, Hackett RL.** 1993. Urinary chemistry of the normal Sprague–Dawley rat. Urol Res **21:**309–312. https://doi.org/10.1007/BF00296826.
37. **Trottier MD, Newsted MM, King LE, Fraker PJ.** 2008. Natural glucocorticoids induce expansion of all developmental stages of murine bone marrow granulocytes without inhibiting function. Proc Natl Acad Sci USA **105:**2028–2033. https://doi.org/10.1073/pnas.0712003105.
38. **Tsubokura Y, Kobayashi T, Oshima Y, Hashizume N, Nakai M, Imatanaka N.** 2016. Effects of pentobarbital, isoflurane, or medetomidine-midazolam-butorphanol anesthesia on bronchoalveolar lavage fluid and blood chemistry in rats. J Toxicol Sci **41:**595–604. https://doi.org/10.2131/jts.41.595.
39. **USDA.** [Internet]. 2015. Public health service policy on humane care and use of laboratory animals. [Cited 16 August 2021]. Available at: https://grants.nih.gov/grants/olaw/references/PHSPolicyLabAnimals.pdf
40. **USDA, Animal and Plant Health Inspection Service.** [Internet]. 2020. USDA Animal Welfare Act and Animal Welfare Regulations. [Cited 21 August 2021]. Available at: https://www.aphis.usda.gov/animal_welfare/downloads/AC_BlueBook_AWA_508_comp_version.pdf
41. **van Herck H, Baumans V, Brandt CJ, Boere HA, Hesp AP, van Lith HA, Schurink M, Beynen AC.** 2001. Blood sampling from the retro-orbital plexus, the saphenous vein and the tail vein in rats: comparative effects on selected behavioural and blood variables. Lab Anim **35:**131–139. https://doi.org/10.1258/0023677011911499.
42. **Weber DK, Danielson K, Wright S, Foley JE.** 2002. Hematology and serum biochemistry values of dusky-footed wood rat (*Neotoma*

fuscipes). J Wildl Dis **38:**576–582. https://doi.org/10.7589/0090-3558-38.3.576.

43. **Weetjens BJC, Mgode GF, Machang'u RS, Kazwala R, Mfinanga G, Lwilla F, Cox C, Jubitana M, Kanyagha H, Mtandu R, Kahwa A, Mwessongo J, Makingi G, Mfaume S, Van Steenberge J, Beyene NW, Billet M, Verhagen R.** 2009. African pouched rats for the detection of pulmonary tuberculosis in sputum samples. Int J Tuberc Lung Dis **13:**737–743.
44. **Wellman ML, Couto CG, Starkey RJ, Rojko JL.** 1989. Lymphocytosis of large granular lymphocytes in three dogs. Vet Pathol **26:**158–163. https://doi.org/10.1177/030098588902600209.
45. **Williams DM, Gillett R, Linder JE.** 1979. Light microscope and electron microscope alkaline phosphatase cytochemistry of rat bone marrow leukocytes. J Histochem Cytochem **27:**665–675. https://doi.org/10.1177/27.2.448058.
46. **Yerroum M, Braconnier F, Chariot P.** 1999. Influence of handling procedures on rat plasma creatine kinase activity. Muscle Nerve **22:**1119–1121. https://doi.org/10.1002/(SICI)1097-4598(199908)22:8<1119::AID-MUS16>3.0.CO;2-S.