BASELINE BIOLOGICAL DATA COLLECTED FROM CHEMICALLY IMMOBILIZED FREE-RANGING BLACK RHINOCEROSES (*DICEROS BICORNIS*) IN ZIMBABWE

Michael D. Kock, B.Vet.Med., M.P.V.M., Raoul du Toit, M.S., Dougal Morton, B.Pharm., Ph.D., Nancy Kock, M.S., D.V.M., and Beverley Paul, M.B.B.S., M.R.C.Path.

Abstract: Biological data were collected from free-ranging black rhinoceroses (Diceros bicornis) that were chemically immobilized for capture and translocation. Physiologic and hematologic data were collected from 31 and 53 rhinoceroses in 1986 and 1988, respectively. Biochemical data, including cortisol, creatine phosphokinase (CPK), lactic dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT), gamma gluteryl transaminase (GGT), alkaline phosphatase (ALP), total protein (TP), albumin, globulin, blood urea nitrogen (BUN), creatinine, glucose, magnesium, phosphorus, calcium, sodium, potassium, chloride, total bilirubin, cholesterol, thyroid stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4), were collected from 53 rhinoceroses in 1988, and TP and cortisol data were collected from 29 rhinoceroses in 1986. Differences (P < 0.05) between adult and subadult rhinoceroses were found for CPK, ALP, GGT, glucose, TP, phosphorus, creatinine, cholesterol, T3, and T4. Significant differences between male and female rhinoceroses were found for CPK, ALP, albumin, globulin, calcium, and phosphorus. Age differences were measured with selected hematologic values, including hemoglobin, mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total white blood cell count and absolute lymphocyte and monocyte counts. Hematologic parameters did not differ between sexes. These results provide baseline reference values for determining deviations from normal health in the black rhinoceros.

Key words: Black rhinoceros, Diceros bicornis, free-living, physiology, hematology, hormone.

INTRODUCTION

The black rhinoceros (*Diceros bicornis*) has declined precipitously in Africa because of habitat loss and poaching. Zimbabwe has the largest remaining population on the continent. Since 1985, >300 black rhinoceroses have been captured and translocated from the high-risk poaching areas of the Zambezi Valley. In 1986, blood samples were collected from 31 of these black rhi-

From the Department of National Parks and Wildlife Management, P.O. Box 8365, Causeway, Harare, Zimbabwe (M. Kock); International Wildlife Veterinary Services, 1850 North Main St., Salinas, California 93906, USA (M. and N. Kock); Zambezi Rhino Project, World Wide Fund for Nature, P.O. Box 8437, Causeway, Harare, Zimbabwe (du Toit); and Faculty of Veterinary Science (N. Kock), and Department of Pharmacy (Morton) and Department of Hematology (Paul), School of Medicine, University of Zimbabwe, P.O. Box MP 167, Harare, Zimbabwe.

noceroses.20 In 1988, samples were collected from >50 black rhinoceroses captured for translocation. In addition, samples were obtained from three black rhinoceroses translocated in 1986 that had escaped from their new sanctuaries, necessitating recapture in 1988. The Zambezi Valley has a diversity of preferred browse plants in both the thicket vegetation along the base of the southern Zambezi escarpment and in small tree savanna woodland and scrub areas that comprise most of the southern half of the Chewore Safari Area. The suitability of this area for the black rhinoceros was reflected in the good health condition of all captured animals.

Limited physiologic, biochemical, and hematologic data are available for free-ranging^{1,4-7,9-11,20} or captive^{2,5,16} black rhinoceroses. Because of the declining status of the species in the wild, limited numbers

in captivity, and a poor record for thriving in captivity, it is important to establish baseline physiologic norms, a recommendation endorsed by the African Rhino Workshop.¹⁶ These data may be essential for understanding and clarifying unsolved and often fatal medical problems experienced by captive animals.^{2,17,19} Such data also will allow investigation of the physiologic response of free-ranging black rhinoceros to capture and relocation and may provide clues to the causes of postcapture mortalities.¹³

During 1986 and 1988, blood samples were collected from rhinoceroses soon after capture. The results of field measurements and laboratory tests performed on these blood samples are presented here.

MATERIALS AND METHODS

All rhinoceroses were captured by darting, either on foot (n = 31 in 1986; n = 10)in 1988) or from a helicopter (1988, n =43), using either etorphine alone (1986, n =23) (M99, 4.9 mg/ml, Rickett and Coleman Pharmaceuticals [Pty.] Ltd., Suidkusweg, 1474 S. Coast Rd., Mobeni 4092, South Africa) or carfentanil alone (1986, n = 8) (Wildlife Laboratories, Inc., Fort Collins, Colorado 80525, USA), 1,20 etorphine plus xylazine (1988, n = 29) (Rompun, 100 mg/ ml, Bayer, Leverkusen, West Germany), or etorphine plus fentanyl (Sublimaze, 40 mg/ ml, Ethnor [Pty.] Ltd., Halfway House, South Africa) plus xylazine (1988, n = 24). ¹⁴ Methods of biological sampling from rhinoceroses in 1986 have been reported.²⁰ In 1988, biological data were collected as soon as the darted animals were located and secured with ropes. 14 Temperature, pulse, and respiration were recorded, and blood samples were collected using an 18-g vacutainer needle (Becton Dickinson, Rutherford, New Jersey 07070, USA) from either the cephalic, tail, or ear vein. Blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes (Monoject, Sherwood Medical, St. Louis, Missouri 63103, USA) for hematology and in lithium heparin and/or serum

tubes for biochemical analyses. Blood glucose was measured immediately following blood collection, using Clinistix (Visidex II, Ames Division, Miles Laboratories, Inc., Elkhart, Indiana 46515, USA). Heparinized or clotted blood samples were centrifuged within 2-6 hr of collection, and the plasma or serum was transferred to 2.0-ml liquid nitrogen storage ampules (Flow Laboratories, P.O. Box 17, Irvine, Ayshire, Scotland) that were frozen immediately in a liquid nitrogen storage cylinder (Thor Industrial Cryogenics, Oxford, England). Red blood cell (RBC) and white blood cell (WBC) counts were measured using a Coulter counter (Coulter Counter Model S Plus, Coulter Electronics Ltd., Northwell Dr., Luton, Bedfordshire, England). Packed cell volume (PCV) was measured using a microhematrocrit method and hemoglobin (Hb) values were determined using the Coulter counter. Thick and thin blood smears were made from the EDTA samples and immediately fixed in methanol (99.5%). Most hematology samples were sent from the Zambezi Valley to Harare on ice within 24 hr of collection for processing by the Hematology Laboratory at Parirenyatwa Hospital. All hematology results reported in this paper, except PCV, are based on results from Parirenyatwa Hospital. Hematology results include data already published from the 1986 capture.20 Laboratory procedures for both 1986 and 1988 were identical.

Biochemical tests were performed between 2 wk and 1 mo after collection, and all samples were maintained frozen in liquid nitrogen or in a -75°C freezer until processed. Cortisol, thyroid stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4) values were determined by radioimmunoassay (Amersham RIA Kits, Amersham International, Bucks, U.K.). For the T3, T4, and cortisol evaluations, samples of rhinoceros serum were assayed then spiked with measured quantitites of the analytes and reassayed; results were within 5%, indicating no serum interference. The TSH assay was not validated. Of the samples in

Age Calf Subadult Sex Adult Totals Male 2 (2.4%)a 5 (6.0%) 32 (38.1%) 39 (46.5%) 4 (4.7%) Female 6 (7.1%) 35 (41.7%) 45 (53.5%) **Totals** 6(7.1%)11 (13.1%) 67 (79.8%) 84 (100%)

Table 1. Age and sex distributions of black rhinoceroses captured, sampled, and translocated in Zimbabwe, 1986 and 1988.

which cortisol was measured (n = 77), 29 were from animals captured in 1986. Total protein (TP), albumin, globulin, creatine phosphokinase (CPK), aspartate transaminase (AST), alanine transaminase (ALT), lactic dehydrogenase (LDH), alkaline phosphatase (ALP), gamma gluteryl transaminase (GGT), total bilirubin, creatinine, blood urea nitrogen (BUN), glucose, calcium, magnesium, phosphorus, and cholesterol were measured using an Electro-Nucleonics Biochemical Analyzer (Altaire, Electro-Nucleonics International Ltd., Adriaan van Bergenstraat 202–208, 4811 S.W. Breda, The Netherlands). Quality control of Altaire procedures utilized Gemcal® Electro-Nucleonics reference serum. Ciba-Corning (Ciba-Corning Diagnostic Corp., Irvine, California 92714, USA) normal and abnormal assay serum were used as controls. Sodium and potassium were measured using a flame photometer (Corning 480 Flame Photometer, Corning Medical and Scientific Ltd., Halstead, Essex, England).

All data collected from the 84 black rhinoceroses were entered onto a spreadsheet (SuperCalc4, Computer Associates International, San Jose, California 95131, USA) using a microcomputer, and a menu-driven database was developed within the spreadsheet. Information was coded for age, sex, and outcome (stress, morbidity, and mortality classifications). Analyses of the data were performed using a statistical graphics program (Statgraphics, Statistical Graphics Corp., Rockville, Maryland 20850, USA). All data are presented as mean ± standard error of the mean and range. Significant

differences in sample results for sex and age were analyzed using Student's *t*-test.³

RESULTS AND DISCUSSION

The age and sex distributions of animals captured are presented in Table 1. Comparison between those rhinoceroses anesthetized with a narcotic alone (n = 31, 1986) versus narcotic/xylazine combinations (n = 53, 1988) revealed higher PCV values for the combination group (45% vs. 42%, P < 0.01). It was not possible to compare biochemical data between the two drug groups as no biochemical tests were performed on the samples collected in 1986.

Physiologic data are presented in Table 2 and are similar to previous reports. 1,4,6,7,9-11 One adult male captured early in the morning with an induction time of 2 min, and therefore minimal compromise, had a rectal temperature of 36.5°C, which may be representative of resting body temperature for the species. Langman reported that temperature change in black rhinoceroses was related directly to behavior and variations in the ambient temperature; periods of depressed rectal temperatures in three captive rhinoceroses occurred between 0600 and

Table 2. Physiologic data from black rhinoceroses chemically immobilized for translocation in Zimbabwe, 1986 and 1988.

Parameter	n	Mean	SEM ^a	Range
Temperature (°C) Respirations/min Pulse (beats/min)	76	38.9	0.11	36.5–41.2
	82	11.4	0.47	5–27
	63	78	2.34	30–160

a Standard error of the mean.

^a Number (percent of total) for each category.

Table 3.	Biochemical	data from	black i	rhinoceroses	chemically	immobilized	for translocation	in Zimbabwe,
1986 and 1	988.							·

Parameter	n	Mean	SEM ^a	Range
Cortisol (µg/dl)	77	2.38	0.12	1.03-6.08
CPK (IU/L)	54	299	20.4	122-992
LDH (IU/L)	44	1,097	56	222-2,394
AST (SGOT) (IU/L)	54	82	3.2	22-132
ALT (SGPT) (IU/L)	54	24	1.16	8-42
ALP (IU/L)	44	217	37.9	51-1,648
GGT (IU/L)	41	19.4	0.60	13–31
Glucose (mg/dl)	54	97.3 (5.4 ^b)	6.7	16-227
Total protein (g/dl)	83	8.4	0.07	7–10
Albumin (g/dl)	49	3.6	0.06	2.7-4.3
Globulin (g/dl)	45	4.6	0.07	3.4-5.7
Total bilirubin (mg/dl)	51	0.43 (7.0°)	0.03	0.12-1.54
Calcium (mg/dl)	52	$11.5 (2.9^{\circ})$	0.15	9.6-14
Magnesium (mg/dl)	52	2.56 (1.05b)	0.07	1.22-4.13
Phosphorus (mg/dl)	52	$3.7 (1.19^{6})$	0.14	1.55-6.2
BUN (mg/dl)	52	$9.9 (3.5^{b})$	0.3	6-15
Creatinine (mg/dl)	53	1.17 (103°)	0.04	0.45 - 1.7
Cholesterol (mg/dl)	51	90 (2.3b)	3.2	42.5-155
Sodium (mEq/L)	35	133.5	1.11	119-151
Potassium (mEq/L)	43	4.39	0.07	3.5-6.7
Chloride (mEq/L)	45	94	0.667	86–104

^a Standard error of the mean.

0800 hr, reaching an average low of 35.8°C at 0630 hr. 15 The rectal temperature in captive white rhinoceroses (Ceratotherium si*mum*) has been reported as 37.5 ± 0.45 °C.²¹ The accuracy of a rectal temperature in an animal as large as the black rhinoceros must be questioned, and our results may not reflect true core body temperature. Both respiration and pulse varied considerably (Table 2), which may represent response to varying degrees of excitement and sedation associated with capture. The adult male with the rapid induction time had a respiratory rate of six respirations/min and a pulse of 66 beats/min, which may be indicative of a nonstressed state. Some subadults received slight overdoses of narcotic and, thus, experienced respiratory depression (four to six respirations/min) at induction.

Biochemical data with comparisons for age and sex are presented in Tables 3 and 4. Cortisol values have not been reported previously in the black rhinoceros, and in this study the range of cortisol was 1.03- $6.08 \,\mu\text{g/dl}$. Cortisol concentrations increase during stress,13 therefore these results are not likely to represent resting values. The mean value for normal unstressed rhinoceroses was 1.69 μ g/dl (n = 27). Both CPK and AST values for these free-living animals were lower than those values reported from captive animals,13,16 although AST values reported from other populations of wild black rhinoceroses were lower than those in Zimbabwe (35 IU/L, n = 9). Age and sex differences were detected for CPK values (Table 4), with adult animals having a higher value than subadults (P < 0.05) and males having a higher value than females (P <0.01). The higher value in adult animals may be due to their active and aggressive nature, and the higher value for males may be related to their comparatively more aggressive temperament. Also, the differences in CPK values by age/sex probably reflected the overall larger muscle bulk of the male.

 $^{^{}b}$ Units = mmol/L

^c Units = μ mol/L.

 Table 4.
 Biochemical data from chemically immobilized black rhinoceroses for significant comparisons by age and sex.

			Age category	gory				Sex category	gory	
•		Adult	Š	Subadult/calf			Male		Female	
Parameter	и	Value	и	Value	P value ^a	и	Value	и	Value	P value
CPK (IU/L)	42	323 ± 25 ^b	12	213 ± 18	<0.05	28	343 ± 32	26	251 ± 21	<0.01
ALP (IU/L)	35	160 ± 18	6	436 ± 157	<0.01	56	270 ± 61	18	140 ± 18	<0.05
GGT (IU/L)	32	20 ± 0.7	6	17.2 ± 0.98	<0.05					
Glucose (mg/dl)	41	107 ± 8 (5.9°)	13	67 ± 11 (3.76°)	<0.05					
Total protein (g/dl)	99	85.1 ± 0.8	16	$8.1 \pm .2$	<0.05					
Albumin (g/L)						25	38 ± 0.7	24	35 ± 0.94	<0.05
Calcium (mg/dl)						27	11.8 ± 0.2	25	11.1 ± 0.24	<0.05
							(2.94°)		(2.78°)	
Phosphorus (mg/dl)	41	3.3 ± 0.12	11	5 ± 0.21	<0.01	27	4.1 ± 0.17	25	3.25 ± 0.2	<0.001
,		(1.07°)		(1.61°)			(1.31°)		(1.05°)	
Creatinine (mg/dl)	40	1.2 ± 0.03	13	0.97 ± 0.05	<0.01					
		(1094)		(864)						
Cholesterol (mg/dl)	38	83 ± 3.1	13	110 ± 6.5	<0.001					
		(2.14°)		(2.85°)						

^{*} Significance determined using paired Student's t-tests. $^{\text{b}}$ Mean \pm SEM.

c Units = mmol/L. d Units = μ mol/L.

Table 5. Thyroid hormone levels in black rhinoceroses chemically immobilized for translocation in Zimbabwe, 1988.

Parameter	n	Mean	SEM ^a	Range
TSH (μIU/ml)	50	1.45	0.03	0.2-1.89
T3 (ng/dl)	51	0.97	0.08	0.7 - 3.7
T4 (pg/ml)	51	0.36	0.04	0.2 - 1.9

a Standard error of the mean.

Baseline values for LDH were relatively high, with a mean of 1,097 IU/L (median: 1,072; range: 222-2,394 IU/L). Values from 320 to 471 IU/L have been reported for captive animals, 16,18 with a mean of 270 IU/L for captive white rhinoceroses.²¹ Free-ranging white rhinoceroses reportedly have LDH values ranging from 335 to 925 IU/L ($\bar{x} =$ 526 IU/L, n = 20).²² Reasons for the relatively high LDH values in recently captured black rhinoceroses were unclear but may have been stress-related; values have been shown to increase in response to chronic stress.¹³ By comparison, LDH in the horse (252 IU/L)⁸ is much lower than LDH values in the black rhinoceros, which are similar to those measured in cattle (1,061 IU/L).8 Differences in LDH levels by age and sex were not detected. Alanine transaminase values were higher (24 IU/L) than those reported from captive rhinoceroses (12–15 IU/ L)¹⁶ or in other free-ranging populations (6 IU/L, n = 9). Again, accurate interpretation of results may be compromised by small sample sizes from both captive and freeranging rhinoceros populations. Alkaline phosphatase values were higher (P < 0.01)in young animals compared with adults (Table 4), a finding that has been reported for other species.8 Although ALP values were consistently higher (P < 0.05) in males than females, the results may have been affected by an age-sex interaction; most of the youngest animals (<4 wk of age) were males. Values for GGT have not been previously reported for free-living black rhinoceroses, but values in this study (19.4 IU/L; range: 13–31 IU/L; n = 41) appeared higher than those reported for the white rhinoceros (7.6)

IU/L; range: 2–13 IU/L; n = 18).²² Adult GGT values were higher than values for subadults (P < 0.05).

When compared with values reported in captive animals. 16 glucose values were higher and spread over a wider range (16.2–227 mg/dl; 0.90-12.6 mmol/L). This probably reflected the effects of capture because glucose is a sensitive indicator of stress in the black rhinoceros,13 and drugs such as xvlazine may affect serum glucose concentration.8 Adults had higher glucose values than subadults (P < 0.05). Total protein values for free-ranging black rhinoceroses were similar to values reported for captive animals, 18 and young animals had lower values than adults (P < 0.05) (Table 4). Albumin values were higher in males than females (P < 0.05), and globulins tended to be higher in females than males (P < 0.10). These free-ranging animals had higher albumin and lower globulins compared with captive counterparts.16 Total bilirubin values in this study (7.0 μ mol/L: 0.4 mg/dl) were high compared with values reported for four captive animals (2.25 μ mol/L; 0.13 mg/dl) (R. A. Kock, pers. comm.) but were similar to those values reported for eight captive animals (8.5 μ mol/L; 0.49 mg/dl).¹⁶

Calcium, magnesium, and phosphorus values were similar to values reported from captive populations. 16 but values were sex dependent; males had consistently higher values than females (Table 4) for calcium (P < 0.05) and phosphorus (P < 0.001). Phosphorus values also were higher in subadults than adults (P < 0.01). Cholesterol values were similar to those previously reported for both captive and free-ranging populations,⁵ but values in young animals were higher than in adults (P < 0.01). In contrast, creatinine values were higher in adults than subadults (P < 0.01), which probably reflected differences in muscle bulk. Creatinine values were similar to those of captive black rhinoceroses.18

There are no baseline TSH, T3, and T4 values in the literature for the black rhinoceros. Subadult black rhinoceroses had

Parameter	n	Mean	SEM ^a	Range
RBC (×10 ⁶ /μl)	84	5.26	0.07	3.6–7.2
Hemoglobin (g/dl)	84	16.1	0.2	10.8-20.6
PCV (%)	87	43	0.55	29-54
Red cell distribution width	48	13.3	0.14	11.2-15.3
MCV (fl)	81	82.5	0.8	59.3-103
MCH (pg)	82	30.9	0.26	23.7-36.4
MCHC	83	37.7	0.44	30.8-61
Platelets ($\times 10^3/\mu l$)	60	210	8.9	102-410
WBC $(\times 10^3/\mu l)$	85	11.5	0.44	4.3-26.2
Neutrophils ($\times 10^3/\mu l$)	81	6.21	0.32	1.8-18.5
Bands $(\times 10^3/\mu l)$	24	0.14	0.019	0.1-0.3
Lymphocytes ($\times 10^3/\mu l$)	86	4.05	0.23	1.0-9.8
Eosinophils ($\times 10^3/\mu l$)	83	0.66	0.054	0.77 - 2.4
Monocytes ($\times 10^3/\mu l$)	82	0.71	0.067	0-3.8
Basophils ($\times 10^3/\mu l$)	11	0.11	0.009	0.05-0.17

Table 6. Hematologic data from black rhinoceroses chemically immobilized in Zimbabwe, 1986 and 1988.

higher T3 (1.37 vs. 0.83 ng/dl; P < 0.01) and T4 (0.56 vs. 0.29 pg/ml; P < 0.01) values than adults; calves (<3 yr old) had the highest values within the subadult group. Other values are reported in Table 5.

Hematologic data are presented in Tables 6 and 7. Of the 84 rhinoceroses, 31 were captured in 1986, and data from these animals have been reported previously. The data reported here were similar to the earlier data. Compared with 1986 results, total WBC counts were lower (1988, $11.5 \times 10^{3}/\mu$ l, range: $4.3-26.2 \times 10^{3}/\mu$ l; 1986, $13.98 \times 10^{3}/\mu$ l, range: $5.6-26.2 \times 10^{3}/\mu$ l). WBC counts were higher when compared with values reported for captive animals. The

eosinophil counts in our study $(0.66 \times 10^3/\mu l)$, range: $0.77-2.4 \times 10^3/\mu l$) were much higher than those reported for captive black rhinoceros $(0.22 \times 10^3/\mu l)$, range: $0.0-0.7 \times 10^3/\mu l$) (R. A. Kock, pers. comm.). This may reflect increased parasitism in the free-ranging group.

Significant differences in hematologic data were not evident between sexes, but subadults had lower hemoglobin values than adults (P < 0.001). Mean corpuscular volume (MCV) (P < 0.05), mean corpuscular hemoglobin (MCH) (P < 0.001), and mean corpuscular hemoglobin concentration (MCHC) (P < 0.05) values for subadults were lower than for adults, and WBC counts

Table 7. Hematologic data for chemically immobilized black rhinoceroses with significant comparisons by age.

		Adult	5	Subadult/calf	
Parameter	n	Value	n	Value	P value ^a
Hemoglobin (g/dl)	65	16.4 ± 0.22^{b}	17	$14.8 \pm 0.57^{\text{b}}$	< 0.001
MCV (fl)	62	83 ± 1	17	79 ± 1.4	< 0.05
MCH (pg)	63	31.5 ± 0.24	17	28.3 ± 0.7	< 0.001
MCHC	64	38.2 ± 0.56	17	36 ± 0.69	< 0.05
WBC ($\times 10^3/\mu l$)	68	10.7 ± 0.46	17	14.8 ± 1.4	< 0.001
Lymphocytes ($\times 10^3/\mu l$)	65	3.47 ± 0.2	16	6.39 ± 0.6	< 0.001
Monocytes ($\times 10^3/\mu l$)	64	0.59 ± 0.05	14	1.21 ± 0.3	< 0.01

^a Significance determined using paired Student's t-tests.

^a Standard error of the mean.

 $^{^{\}text{b}}$ Mean \pm SEM.

in subadults were higher than in adults (P < 0.001). Absolute cell counts also differed between age groups; subadults had higher lymphocyte (P < 0.0001) and higher monocyte counts (P < 0.01) (Table 7).

Collection of physiologic, biochemical, hormonal, and hematologic data from free-living male and female black rhinoceroses provides valuable baseline information. These data have assisted in the overall medical management of this endangered species from capture through translocation.¹³ The biological database established in 1988 is currently being expanded to include data from more than 100 Zimbabwean animals.

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