CLINICAL PATHOLOGY PARAMETERS IN WHITE, BLACK AND NORTHERN WHITE RHINOS

J van Heerden[a], R H Keffen[b], F Kuhn[c], P Rogers[d], P Morkel[e], N Atalia[f], J P Raath[g] and D J Kernes[h]

INTRODUCTION

Laboratory-determined biological parameters are extensively employed in the diagnosis of disease in man and animals, in preventive medicine and as management tools. Reference ranges that serve as a guide to the veterinary clinician in the evaluation of parameters obtained from specific animals, should ideally be obtained from a population of clinically healthy and productive animals. A wide variety of tissues and body fluids can be subjected to laboratory assay. Blood may, however, be the easiest specimen to collect from rhinos immobilised under field conditions. The serum obtained from clotted blood can be subjected to a variety of investigations e.g., blood chemistries, hormonal assays and serological assays. Biological parameters determined on specimens obtained from relatively small numbers of free-living animals under field conditions should however at best be regarded as relatively crude estimates of the normal range. The sex, age and condition of animals, time of day specimens were collected, activity of animals prior to darting, the type and dosage of drug(s), time to immobilisation, time between darting and collection of specimens, handling of specimens and method and time of storage of specimens are some of the factors that may have an influence on haematological, blood chemical and hormonal parameters. It is also impossible to establish the health status of free-ranging animals prior to immobilisation. This paper report on selected baseline blood chemical and haematological parameters for free-ranging white, northern white and black rhinos as well as the diagnostic value of serum progesterone assays in these animals.

METHODS

All blood specimens in this investigation were collected when opportunities arose when rhinos were captured for either translocation or for temporary transfer to bomas prior to public auctions. Blood specimens were collected from white rhinos (*Ceratotherium simum*)[8] (n = 20) (8♂♂, 12♀♀; 16 adults and 4 subadults) immobilised with etorphine hydrochloride, azaperone and fentanyl as previously reported. Animals were immobilised between 07:00 and 11:05 in the mornings. Serum specimens were collected within 5 to 80 min of darting of animals. Two animals that respectively received 2

[a] Private Veterinary Specialist Practitioner, P O Box 12900, Onderstepoort, 0110, Rep. of South Africa

[b] Private Practitioner, Mogwase

[c] Dept of Chemical Pathology, Faculty of Medicine, Medical University of Southern Africa

[d] Veterinarian, Natal Parks Board, Mtubatuba

[e] National Parks Board, Kimberley

[f] Institut Zairois pour la Conservation de la Nature, Parc National de la Garamba, Zaire

[g] National Parks Board, Skukuza, Rep. of South Africa

[h] Department of Haematology, Faculty of Medicine, Medical University of Southern Africa

and 4 darts, were bled 69 and 80 min later. If the latter data are omitted the average time between darting and bleeding were 21,6 min. Blood specimens were centrifuged within 1,5 h of collection and the serum stored at -4°C until analysis was performed. Blood specimens from white rhinos (n = 63) for hormone assays were collected at the same time as well as with other routine capture operations.

Blood specimens were collected from black rhinos (*Diceros bicornis*) (n = 13) (5♂♂, 8♀♀) immobilised with 2,5-2,7 mg etorphine hydrochloride, 12-17 mg of fentanyl and 50-75 mg of azaperone. These black rhinos were immobilised on average within 7 min and 8 s and the average time to collection of blood specimens was 12 min and 8 s. Blood specimens were centrifuged within 1,5 h of collection and the serum stored at -4°C until assayed. Blood specimens from black rhinos (n = 21) for hormone assays were collected at the same time as well as during other routine capture operations.

Blood specimens were collected from northern white rhinos (*Ceratotherium simum cottoni*) (n = 6) (3♂♂, 3♀♀) immobilised with 2,8-3,8 mg etorphine hydrochloride, 10-16 mg detomidine and 1 500 IU hyaluronidase. The average time to immobilisation was 13 min 9 s and the average time between darting and collection of blood specimens was 41 min 29 s.

Blood specimens for determination of haematological parameters were collected in EDTA vacutainers from black (n = 12) and white rhinos (n = 9) immobilised with etorphine hydrochloride (*ca* 4 mg) and azaperone (*ca* 200 mg). The ages of these black rhinos (4♂♂, 8♀♀) ranged from 4,5 to 20 years.

Serum specimens were analysed for chemical substances as listed in Table 1 according to described laboratory methods[7,8]. Serum concentrations of cortisol, progesterone and 17-ß-oestradiol were determined by using commercial kits [Clinical Assays Gamma Coat™(^{125}I) Cortisol Radio-immunoassay Kit, Division of Travenol Laboratories; Coat-A-Coat® Progesterone, Diagnostic Products Corporation; Coat-A-Count® Estradiol, Diagnostics Products Corporation) prepared for use in human diagnostics. Red and white blood cell counts as well as haemoglobin parameters, packed cell volume, mean cell haemoglobin, mean cell haemoglobin concentration as well as platelet count were determined with a Coulter counter [Coulter Counter Model T 890 (Vetpack included), Coulter South Africa]. Differential counts were obtained by examination of stained blood smears.

Observations (by the authors or owners of the rhinos) continued for several months after collection of blood specimens, to establish the presence or absence of offspring.

RESULTS

The results of blood chemical and haematological investigations are presented in Tables 1-3. Published results of a southern African investigation[5] are also shown in Tables 1 and 2.

Considerable variation occurred in serum concentrations of 17-ß-oestradiol in black, white and northern white rhinos that ranged from less than 70 to 1340 pmol/ℓ. No correlation between pregnancy or non-pregnancy and concentrations of 17-ß-oestradiol could be established. Pregnant white and black rhinos had serum progesterone concentrations ranging respectively from 4,2 to more than 127 nmol/ℓ and from 47 to more than 127 nmol/ℓ. Nonpregnant white, black and northern white rhinos had serum progesterone concentrations ranging from 0,3 to 4 nmol/ℓ. Table 4 presents serum progesterone concentrations at different stages of pregnancy in rhinos where the birth date could be established with reasonable certainty (gestation periods of 16 and 15 months respectively are assumed for white and black rhinos).

DISCUSSION

The results of blood chemical, haematological and hormonal investigations are presented with full realisation of the lack of standardisation of factors that could have affected these results when the

blood specimens were collected. The primary concern of involved teams during these capture operations that were undertaken under field conditions in often difficult terrain, was, however, the safety and health of the rhinos. Collection of blood specimens for laboratory investigation was merely a by-product of the procedure. However, standardisation of procedures for rhino immobilisation under field conditions will probably never be achieved. Despite all the possible shortcomings, the values of the different parameters are generally in agreement with values reported for black rhinos by Kock *et al.*[5] and could probably be accepted as a guideline of ranges of these parameters in free-ranging apparently healthy white, black and northern white rhinos.

The values of certain parameters differ considerably from the normal range accepted for domesticated perissodactyls and should be noted by veterinary clinicians. The total serum protein concentration is considerably higher than that of horses. The albumin concentration may be lower than that reported for horses. The bulk of the serum proteins consists of globulins and concentrations may range from 40 to 70 g/ℓ. This concentration of globulins appears to be normal for rhinos and should not be interpreted as indicative of for example liver disease, chronic inflammation or immune-mediated disease. The electrophoretogram which has been reported usually indicates broad "peaks" in presumably alpha, beta and gammaglobulins.

Serum concentrations of sodium and chloride are lower than values reported for most domesticated mammals and should not be evaluated as indicative of for example excessive loss of electrolytes due to diarrhoea or chronic renal disease. Kock *et al.*[5] described age and sex differences of a number of blood chemical parameters in black rhinos: adults had higher blood glucose, creatinine and serum protein concentrations than subadults. Males had higher serum albumin, phosphorous, and calcium concentrations than females. Subadults had higher serum phosphorous and cholesterol concentrations than adults.

Considerable variation occurred in the serum activities of the various enzymes measured and the average serum activity of lactate dehydrogenase is considerably higher than what has been reported for horses. The average serum activities of all enzymes reported here are also lower than averages reported for black rhinos in Zimbabwe. The latter study however, included a considerably larger group of animals which does not allow for meaningful comparison of these results with results obtained in this paper. Kock *et al.*[5] also found higher serum activities of creatine kinase in adult animals and in males, lower serum activities of alkaline phosphatase in adults and higher serum activities of gammaglutamyltransferase in adults. Changes in serum activities of creatine kinase and aspartate transaminase have been associated with aggressive animals and changes in blood glucose and cortisol concentrations related to capture stress[3,4].

Despite the relatively small number of black rhino specimens subjected to haematological investigation, the values of the different parameters are remarkably similar to those reported for black rhino by Kock *et al.*[5]. The diagnostic importance of haematological data is well known and Kock *et al.*[4] related changes in certain parameters to capture, transport and confinement.

Serum progesterone concentrations in this investigation were found to be reliable indicators of pregnancy in black and white rhinoceroses but the wide range of serum concentrations observed would probably imply limited use in determination of the stage of pregnancy. More information on time of birth in animals in which serum progesterone concentrations in excess of 4 nmol/ℓ have been found, may contribute to our understanding of the possible significance of observed serum progesterone concentrations. The wide fluctuation found in concentrations of metabolites of progesterone in the urine of different rhinos[1] as well as observed differences in urinary concentrations of hormone metabolites between different species of rhino[6] probably imply similar differences in serum hormonal concentrations. This probably even further belittles the value of single-serum progesterone concentration determinations. From our data and from data obtained from urinary steroid evaluations in black rhinos[6], it does however appear as if serum progesterone concentrations are very low during the first part of pregnancy, that concentrations may gradually rise during midgestation and thereafter until shortly before partus when there is a sharp decline. The possible influence of capture- and boma-stress on serum progesterone concentrations[8] has also not been investigated.

Single serum 17-ß-oestradiol concentrations could not be related to pregnancy or non-pregnancy in the different rhino species in this investigation. Urinary estrone conjugate concentrations could likewise not be related to oestrus, postoestrus or early, mid- or late-gestation samples in black rhinos[6]. All phases of the reproductive cycle of the Indian rhinoceros could however be characterised by discrete concentrations of urinary estrone sulphate and pregnanediol-3-glucuronide[2]. Urinary hormone assays at this stage however appears to be an impractical method of investigation of the reproductive state of rhinos in South Africa.

The ability to confirm pregnancy in rhinos has many possible advantages: Known breeders could be identified and either withdrawn from the sale or offered for sale as pregnant animals. Pregnant animals should fetch higher prices at game sales. Pregnant animals should be considered at increased risk for chemical immobilisation, should receive optimal care when held in bomas or may even preferably be released. White rhinos in particular often do not adapt easily to captive conditions and pregnant animals are likely to abort. Recently 7 out of 9 white rhinos diagnosed as pregnant (based on serum progesterone concentrations) aborted and one gave birth to an apparently premature calf which died after 7 days. Known pregnant animals could also be released into the most appropriate habitat. Lengthy potential stressful translocation and boma-training programmes should preferably not be undertaken in pregnant animals.

From limited observations and investigations to date it appears as if pregnancy should be assumed in an adult cow captured without a calf or if the calf is older than 18 months. Serum specimens should be collected at the time of capture for progesterone assay and if pregnancy is suggested, the cow should either be released (to prevent abortion or to avoid the calf been borne in captivity which may seriously jeopardise its chances of survival) or management should be upgraded and maintained at optimal levels at all times. A pregnant animal that does not want to feed in captivity is likely to abort.

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Table 1: Blood chemical parameters for white, black and northern white rhinos as well as reported values for black rhinos

	White rhino n = 20		Black rhino n = 13		Northern white n = 6		Black rhinos** n = 35-77	
	x	sd	x	sd	x	sd	x	range
Albumin g/ℓ	26,1	3,7	35	1,7	31,3	1,2	36	27-43
Alanine transaminase U/ℓ	8,6	3,7	15	14,4	9,3	0,5	24	8-42
Alkaline phophatase U/ℓ	127	33,2	95	35,4	60,8	19,8	217	51-1 648
Aspartate dehydrogenase U/ℓ	40	14,6	52,5	15,3	53,6	13,2	82	22-132
Bilirubin µmol/ℓ	nd	nd	7,7	1,8	nd	nd	7	2,04-26,08
Calcium mmol/ℓ	nd	nd	2,85	0,14	2,50	0,04	2,90	2,4-3,5
Chloride mmol/ℓ	94,2	3,05	93	3,06	95,5	3,27	94	86-104
*Creatine kinase U/ℓ	48	14,1	143	42	147,5	81,4	204	122-992
Creatinine µmol/ℓ	nd	nd	93	14,6	nd	nd	103,4	39,7-150,2
Cholesterol mmol/ℓ	nd	nd	3,43	0,64	1,55	0,39	2,33	1,1-4,01
Cortisol nmol/ℓ	2[illegible]2	32,4	110	37	nd	nd	65,7	28,4-167
*Gammaglutamyl transferase U/ℓ	7,6	2,8	14,6	2,1	11,8	4,7	19,4	13-31
Glucose mmol/ℓ	nd	nd	6	1,2	nd	nd	3,8	0,9-12,7
Lactate dehydrogenase U/ℓ	526	126	695	146	702	101	1 097	222-2 394
Magnesium mmol/ℓ	nd	nd	1,03	0,11	nd	nd	1,05	0,5-1,69
Phosphorous mmol/ℓ	nd	nd	1,56	0,29	nd	nd	1,19	0,5-2,0
Potassium mmol/ℓ	5,4	2,6	5,09	0,96	4,31	0,58	4,39	3,5-6,7
Sodium mmol/ℓ	129,6	4,2	131	3,4	133,7	3,82	133,5	119-151
Total proteins g/ℓ	92,7	9,0	95,0	5,6	76,2	4,8	84	70-100
Triglycerides mmol/ℓ	nd	nd	0,94	0,17	nd	nd	nd	nd
Urea mmol/ℓ	nd	nd	3,3	0,6	nd	nd	2,49	1,5-3,7

*3 rhinos with creatine kinase concentrations of 200, 559 and 1800 U/ℓ as well as 2 rhinos with GGT concentrations of >3 350 and 165 were excluded.

**Values reported by Kock *et al.*[5]., changed to SI units by authors

Table 2: Haematological parameters of black rhinos (mean, standard deviation and range) as well as reported parameters[6]

Parameter	mean	sd	range	X[000]
Red cell count (x10^6/ℓ)	5,74	0,49	4,99-6,54	5,26
Haemoglobin (g/ℓ)	16,52	1,59	14,3-19,1	16,1
Packed cell volume	43,9	4,41	37,5-51	43
Mean corpuscular volume (fℓ)	76,4	5,49	71,2-83,3	82,5
Mean corpuscular haemoglobin (pg)	28,7	1,29	27,4-30,9	30,9
Mean corpuscular haemoglobin concentration (g/dℓ)	37,6	1,73	36,7-38,7	37,7
Platelets (x10^9/ℓ)	293	92	158-467	210
White cell count (x10^9/ℓ)	11,33	2,31	6,9-15,4	11,5
Neutrophils %	50	18,71	23-82	54*
Immature neutrophils %	0,25	-	-	1*
Lymphocytes %	40	17	11-67	35*
Monocytes %	3,75	-	1-10	6*
Eosinophils %	3,9	-	0-10	5*
Basophils %	1,08	-	0-6	0,9*

[000]baseline parameters for free-ranging black rhinos in Zimbabwe, as published by Kock *et al*[6].

*values changed by the authors to percentages

Table 3: Haematological parameters of white rhinos (n = 9) [mean, standard deviation and range]

Parameter	mean	sd	range
Red cell count (x10^9/ℓ)	6,17	0,49	5,6-6,96
Haemoglobin (g/ℓ)	13,78	1,32	12,1-15,9
Packed cell volume	37,6	3,98	33-43,4
Mean corpuscular volume	61	5,26	55-70,9
Mean corpuscular haemoglobin (pg)	22,37	1,81	20,2-25,6
Mean corpuscular haemoglobin concentration (g/dℓ)	36,8	0,75	35,4-38
Platelet count (x10^9/ℓ)	483	152	255-696
White cell count (x10^9/ℓ)	15,14	2,55	11,1-19,5
Neutrophils (%)	26,7	9,1	13-38
Lymphocytes (%)	61,2	10,7	48-79
Monocytes (%)	3,4	-	1-10
Eosinophils (%)	5,5	-	1-12
Basophils (%)	0,7	-	0-2

Table 4: Progesterone concentrations in white and black rhinos at different stages of pregnancy

Species	Month of pregnancy	Progesterone nmol/ℓ	Comments
White rhino	2?	4,2	Aborted a 0,3 kg male foetus 3 months later
	4	6	
	4?	6	Aborted a 2 kg male foetus 3 months later
	4	5,1	
	4	10.5	
	5	5,1	
	7	88	
	8?	29	Aborted a 13 kg female foetus 2 months later
	9	31	Calved 6 months later; 33 kg female calf that died
	10	69	
	13	68	
	13?	60,1	Calved a month later; 38 kg male calf that died
	13	75	
	15	70	Calved 2 weeks later; 48 kg male calf that died within 48 hours
	15	54	
	15	58	
	16	40	Calf died 7 days after birth; body mass 50 kg*
Black rhino	6	50	
	8	61,3	
	9	>127	
	14	47,2	

* Normal body mass at birth appears to be *ca* 50 kg