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USE OF HYALURONIDASE AND INCREASED ETORPHINE (M99) DOSES TO IMPROVE INDUCTION TIMES AND REDUCE CAPTURE-RELATED STRESS IN THE CHEMICAL IMMOBILIZATION OF THE FREE-RANGING BLACK RHINOCEROS (*DICEROS BICORNIS*) IN ZIMBABWE

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Abstract: Twenty free-ranging black rhinoceroses (*Diceros bicornis*) were immobilized in 1991 and 14 in 1990 using mixtures of etorphine (mean dose = 3.9 ± 0.18 mg; median = 4 mg), xylazine (100 mg), and hyaluronidase (4,500 IU). Induction times ($\bar{x}$ ± SEM) were 7.0 ± 1.2 min (median = 5 min) and 3.8 ± 0.48 min (median = 3 min), respectively. Mean durations of immobilizations were 180 ± 20 min and 247 ± 35.6 min, respectively. When compared with immobilizations carried out in 1988 using less etorphine (3.0 mg) mixed with 100 mg of xylazine alone, the increase in M99 dose and the addition of hyaluronidase significantly lowered induction times and reduced stress ($P < 0.05$) as characterized by physiological (temperature and respiratory rate) and biochemical (glucose, creatine phosphokinase, aspartate transaminase, and lactic dehydrogenase) measures. Twenty-four percent of the rhinoceroses were classified as stressed in the M99/xylazine and hyaluronidase group compared with 57% in the M99/xylazine group. In addition, improvements in reversal times in 1991 were significant ($P < 0.01$) using naloxone (50 mg) combined with 6 mg diprenorphine (M50-50) (mean reversal time = 78 ± 7 sec) compared with the 1988 and 1990 use of M50-50 alone (mean reversal time = 161 ± 13 sec). The results indicate the advantage of increasing M99 doses and using hyaluronidase in reducing induction times and capture-related stress, as well as improving reversal times with the addition of naloxone.

Key words: *Diceros bicornis*, black rhinoceros, immobilization, etorphine, xylazine, hyaluronidase, nalorphine, naloxone, diprenorphine, stress.

INTRODUCTION

Capture and translocation of the black rhinoceros (*Diceros bicornis*) has been undertaken in Zimbabwe since 1986 in response to the poaching threat from Zambia.[2,6] Indirect capture mortalities have been unacceptably high.[6,7] Although the black rhinoceros appears to be quite resistant to peracute and acute capture stress, the latent effects of stress associated with capture, boma (holding pen) confinement, and translocation may ultimately predispose the black rhinoceros to mortality.[7] The addition of hyaluronidase to immobilizing drugs has improved absorption times in other species.[4,5,9] Its addition to immobilizing drugs for the black rhinoceros, along with an increase in the M99 dose, was done in an attempt to reduce induction times and improve biological measures indicative of stress.

MATERIALS AND METHODS

Fourteen black rhinoceroses in 1990 and 20 in 1991 were captured and relocated from the Chete Safari Area (17°15'S, 27°45'E), near Lake Kariba, to other areas in Zimbabwe and for ex situ captive breeding programs. Capture of the 34 rhinoceroses was carried out on the ground as previously reported.[6] All rhinoceroses were darted with a mixture of etorphine (mean dose = 3.9 ± 0.18 mg) (M99, 4.9 or 9.8 mg/ml; C-Vet, Ltd., Minster House, Bury St. Edmunds, Suffolk, U.K.) and 100 mg xylazine (Rompun, 100 mg/ml; Bayer, Leverkusen, Ger-

From the Department of National Parks and Wildlife Management, P.O. Box 8365, Causeway, Harare, Zimbabwe; and International Wildlife Veterinary Services, 1850 North Main Street, Salinas, California 93906, USA.

many) with the addition of 4,500 IU hyaluronidase (Hyalase, 1,500 IU dry powder per ampoule; Fisons Pharmaceuticals, Pty., Ltd., 1624 Chloorkop, South Africa). In 1990, the opioid was reversed after the animals were transported on a sled to the holding bomas with 12 mg diprenorphine (6 mg i.v. and 6 mg i.m.) (M50-50, 12 mg/ml; C-Vet, Ltd.). In 1991, reversal was achieved using 50 mg naloxone i.v. (Naloxone, 50 mg/ml; Wildlife Laboratories, Inc., Fort Collins, Colorado 80524, USA) and 6 mg diprenorphine i.m.

Darting procedures were identical for the 1990 and 1991 capture operations. Projectile syringes were loaded on the morning of capture or just before a suitable animal was spotted. Etorphine was added first, followed by xylazine mixed with hyaluronidase. The syringe volume was topped off with sterile water. Any darts unused on the day of preparation were refrigerated overnight and discarded after 48 hr. Either Lowndes capture darts (3–4-ml volume with 47–63- × 5-mm collared needles) fired from a Lowndes compressed air rifle (Don Lowndes, D.L. Tranquilizer Systems, Ashes Farm, Fenny Bentley, Derbyshire, U.K.) or Palmer Capchur darts with Simmons tailpieces (3 ml with 46- × 5-mm collared needles) (Palmer Chemical and Equipment Co., Inc., Douglasville, Georgia 30134, USA) fired from a model 171C Pneudart powder charge rifle (Pneudart, Inc., Williamsport, Pennsylvania 17703, USA) were used. Most rhinoceroses were darted in the caudal thigh, shoulder, or neck ($n = 29$), although some were darted in the abdomen or chest ($n = 5$). Several rhinoceroses were given 200 mg of doxapram HCl (Dopram-V, A. H. Robins, Richmond, Virginia 23220, USA) i.v. ($n = 14$) or 5–10 mg nalorphine (Nalorphine hydrobromide, Centaur Labs, Pty., Ltd., Johannesburg, South Africa) i.v. ($n = 9$) immediately after immobilization to counteract respiratory depression. The nalorphine was also used for partial anesthetic reversal of animals so that they could be walked out of gullies, rocky areas, or riverbeds ($n = 7$). Walking the rhinoceroses was accomplished by placing a rope around the rear horn and lower jaw and ropes around the distal hind legs, which acted as a "brake" when going down inclines or moving at excessive speeds. After placing the ropes, each rhinoceros was given between 30 and 50 mg nalorphine i.v., and when ear movement indicated partial reversal (~2–5 min later), an electric cattle prod was directed around the perineal area, which caused the animals to start moving. Direction was maintained with the head rope and two individuals walking alongside the animal at the shoulders.

Procedures for locating, collecting biological data from, and loading and transporting the immobilized rhinoceroses have been described previously,[6,7] and blood sample collection, processing, and storage were identical to that in previous reports.[7,8] The majority of the first blood samples (sampling period 1)[7] were collected soon after immobilization (mean time from immobilization = 18 ± 2 min), prior to any medication or tranquilizer being administered. The second blood sample (sampling period 2) was collected prior to anesthetic reversal (mean time postimmobilization = 200 ± 30 min), with most collected after the administration of long-acting neuroleptics. Serum cortisol, glucose, creatine phosphokinase (CPK), aspartate transaminase (AST), and lactic dehydrogenase (LDH), levels were measured in the laboratory and validated as previously reported.[7,8]

Each rhinoceros was classified into an outcome category at capture, the classification system was identical to that used previously.[6] An animal experiencing rapid induction (within 6–13 min after darting) without complications was placed in the normal outcome category. If a rhinoceros experienced a prolonged chase before darting; some agitation and excitement during darting; or an extended induction period (>13 min after darting) with significant struggling, sweating, or signs of exhaustion including open mouthed breathing or if immobilization generally was poor, the animal

was categorized as stressed. All rhinoceroses were given various combinations of long-acting tranquilizers (neuroleptics), including zuclophenthixol acetate (Clopixol-Acuphase, 50 mg/ml; H. Lundbeck A/S, DK-2500 Copenhagen, Denmark) and perphenazine enanthate (Trilafon, 100 mg/ml; Sherag, Pty., Ltd., Isando, Transvaal, South Africa) to reduce aggression, produce calming, and prevent self-inflicted trauma during boma confinement and prior to translocation.

Induction times and biological measures were compared with 27 animals captured in 1988,[6,7] when only M99/xylazine were used. Data were analyzed using a statistical graphics program (StatGraphics, Statistical Graphics Corp., Rockville, Maryland 20850, USA). Specific statistical tests were applied following exploratory data analysis, including one-way analysis of variance (ANOVA) (e.g., drug combination vs. induction times, and distance moved; effects of the different drug combinations on temperature, respiration, pulse, serum cortisol, glucose, CPK, AST, LDH levels, and reversal times) and the chi-square test for independence (e.g., outcome classification vs. drug combination used; muscle relaxation vs. drug combination used; tractability vs. drug combination used). Because the raw data for induction times, distance moved, and CPK and LDH values were distributed log normally, logarithmic transformation was performed prior to statistical analyses.[3] All data are presented as $\bar{x} \pm$ SEM.

Total induction time refers to the time from dart impact to the time when the animal became immobile, including both recumbency and standing sedation. Muscle relaxation refers to the degree of muscle tremor and/or rigidity observed. Tractability refers to the degree of narcotic sedation and, therefore, the ability of the capture team to safely handle the rhinoceros without further sedation. The total time of reversal refers to the time from administration of the antagonist to the time when the animal stood up.

RESULTS AND DISCUSSION

Of the 34 rhinoceroses captured in 1990 and 1991, 27 were adults, two were subadults (>3 yr and <5 yr old), and five were calves (<3 yr old); of those captured in 1988 (n = 27), 23 were adults, three were subadults, and one was a calf.

Table 1 summarizes the results of immobilizations with M99/xylazine and hyaluronidase for 1990 and 1991, with comparisons with the M99/xylazine procedure used in 1988. The induction time ($\bar{x} \pm$ SEM) for the total number of animals immobilized in 1990 was 7.0 ± 1.2 min (median = 5 min). Prolonged induction occurred in two animals because of poor dart placement and dart failure. In 1991, a further reduction in mean immobilization times was achieved (3.8 ± 0.48 min; median = 3.0 min). The M99/xylazine/hyaluronidase combination resulted in significantly reduced induction times ($P < 0.0005$) compared with those of M99/xylazine alone (Table 1, Fig. 1). Use of M99/xylazine alone resulted in a mean induction time of 24 ± 4.2 min (median = 16.0 min). The removal of outliers (n = 7) (e.g., extended induction times occurred when lengthy tracking was needed for location, etc.) reduced mean induction time to 17 ± 2.5 min. Significant differences were also noted in how far the animals moved after darting between 1988, 1990, and 1991 (Table 1, Fig. 2). In 1990 and 1991, several rhinoceroses became immobile 120–500 m from the ambush site, reflecting very rapid development of incoordination, ataxia, and final recumbency.

In 1990, rhinoceroses remained recumbent for 180 ± 20 min, with an average transport time of 117 ± 13.4 min. In 1991, animals were recumbent for 247 ± 36 min (median = 190 min), with an average transport time of 107 ± 11 min. The longer recumbency time in 1991 is skewed by the capture of four animals in difficult terrain, which resulted in prolonged anesthesia (>8 hr). The reduced transport time reflects an improvement in the bush roads used to transport animals to the holding bomas. Re-

Table 1. Comparison of measures monitored in black rhinoceroses immediately after chemical immobilization with either M99/xylazine and hyaluronidase (1990 and 1991) or M99/xylazine alone (1988) in Zimbabwe.

Measure	M99/xylazine/hyaluronidase 1990 n	$\bar{x} \pm$ SEM	1991 n	$\bar{x} \pm$ SEM	M99/xylazine n	$\bar{x} \pm$ SEM
Induction time (min)	14	7.0 ± 1.2[a]	20	3.8 ± 0.5[a]	27	24 ± 4.2[a]
					22	17 ± 2.5[b]
Distance moved during induction (km)	14	1.3 ± 0.33[a]	20	0.7 ± 0.1[a]	25	2.9 ± 0.5[a]
Total duration of anesthesia (min)	14	180 ± 20	20	247 ± 36	27	152 ± 11
Total transport time (min)	14	117 ± 13	20	107 ± 11.4	27	80 ± 6
Temperature (°C)	14	37.6 ± 0.23[a]	20	37.7 ± 0.2[a]	27	38.8 ± 0.19[a]
Respiration (breaths/min)	14	7 ± 0.9[a]	20	7 ± 0.5[a]	27	10 ± 0.8[a]
Pulse (beats/min)	14	66 ± 4.1	20	76 ± 3.7	21	77 ± 5.2
Cortisol (μg/dl)	14	1.67 ± 0.25	19	2.5 ± 0.18	27	2.12 ± 0.13
Glucose (mg/dl)	14	62.5 ± 6.6	19	57.6 ± 5.4	27	102.9 ± 6
CPK (IU/L)	14	214 ± 38[a]	19	299 ± 111	27	348 ± 32[a]
AST (IU/L)	14	96 ± 7[a]	19	67 ± 4[a]	27	91 ± 4.2[a]
LDH (IU/L)	14	738 ± 66[a]	19	392 ± 30[a]	23	1136 ± 57[a]

[a] Means significantly different ($P < 0.05$); see text.
[b] Outliers removed.

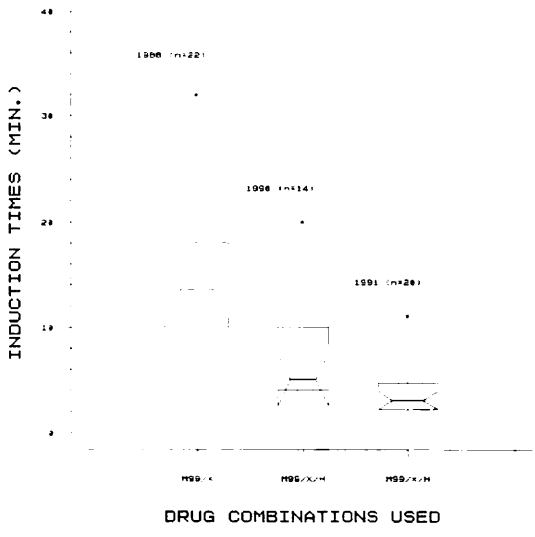

Figure 1. Notched box and whisker plots displaying the relative data distribution of induction times (min) for black rhinoceroses immobilized with either M99/xylazine/hyaluronidase or M99/xylazine alone. The median is portrayed by the horizontal line segment within the box. The notches provide an approximate 95% test of the null hypothesis that the true medians are equal. The whiskers represent the tails of the distribution and the outside values (*) are considered outliers.

versal of the opioid with naloxone i.v. and M50-50 i.m. produced smoother, more rapid returns to consciousness (mean reversal time = 78 ± 7 sec), without the initial staggering and incoordination seen with M50-50 i.v. alone (mean reversal time = 172 ± 27 sec).

Significant differences were seen in some physiological measures between the 1988 and 1990–1991 animals (Tables 1, 2); differences in body temperature ($P < 0.0001$) and respiration ($P < 0.009$) were most notable. The M99/xylazine/hyaluronidase combination resulted in lower body temperatures (Fig. 3), even lower than baseline values recently reported.[7] Because the 1990 captures occurred during the hot winter months, when average temperatures approached 36°C, compared with the 1988 captures in the cool winter months, when average temperatures approached 26°C, this difference in body temperature is impressive. The significantly lower respiratory rates in animals given M99/xylazine/hyaluronidase probably reflected the increased opioid dose.

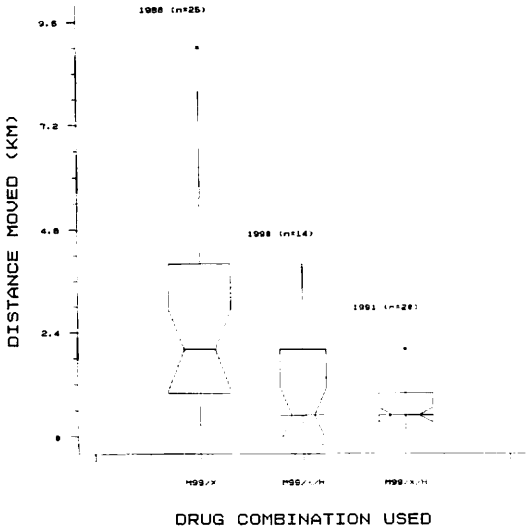

Figure 2. Notched box and whisker plots demonstrating the reduction in distances run by darted black rhinoceroses immobilized with M99/xylazine/hyaluronidase (1990 and 1991) and M99/xylazine alone (1988).

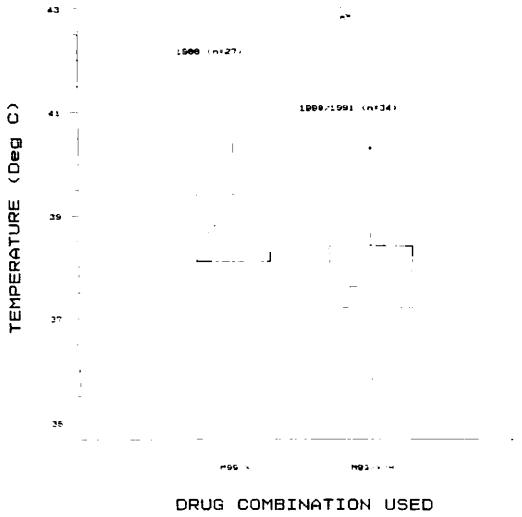

Figure 3. Comparison of relative data distributions of body temperatures (°C) measured following immobilization of black rhinoceroses with either M99/xylazine/hyaluronidase (combined 1990/1991 data) or M99/xylazine alone (1988).

Eight rhinoceroses immobilized with M99/xylazine/hyaluronidase were classified as stressed (24%) compared with 15 (57%) in the M99/xylazine group. A statistical association was confirmed for outcome classification and the different drug combinations ($P < 0.05$); those animals immobilized with M99/xylazine/hyaluronidase were less likely to be classified as stressed.

Cortisol and glucose are sensitive indicators of stress in the black rhinoceros.[7] Cortisol values for the M99/xylazine/hyaluronidase groups did not differ significantly ($P > 0.05$) from values for the M99/xylazine group. Initial comparisons of cortisol values between the 1988 and 1990 immobilizations were skewed by two subadults in the M99/xylazine/hyaluronidase group that had much higher cortisol values (2.7 μg/dl and 2.3 μg/dl) than average (1.6 ± 0.2 μg/dl). Calves and subadults are usually darted successfully after their mothers because they tend to guard the recumbent adult and can

Table 2. Serum cortisol, glucose, CPK, AST, and LDH values collected after sled transport and prior to reversal of M99 in black rhinoceroses chemically immobilized with either M99/xylazine and hyaluronidase (1990 and 1991) or M99/xylazine alone (1988) in Zimbabwe.

Measure	M99/xylazine/hyaluronidase[a]		M99/xylazine		*P*-value[b]
	n	$\bar{x}$ ± SEM	*n*	$\bar{x}$ ± SEM	
Cortisol (μg/dl)	33	4.0 ± 0.2	24	3.5 ± 0.2	>0.05
Glucose (mg/dl)	31	146 ± 6.5	25	182 ± 7.3	<0.0007
CPK (IU/L)	28	336 ± 42	25	437 ± 49	<0.06
AST (IU/L)	31	80 ± 4.7	25	106 ± 5.6	<0.002
LDH (IU/L)	34	753 ± 38	25	928 ± 42	<0.004

[a] Combined data from 1990 and 1991 captures.
[b] Significance determined using ANOVA.

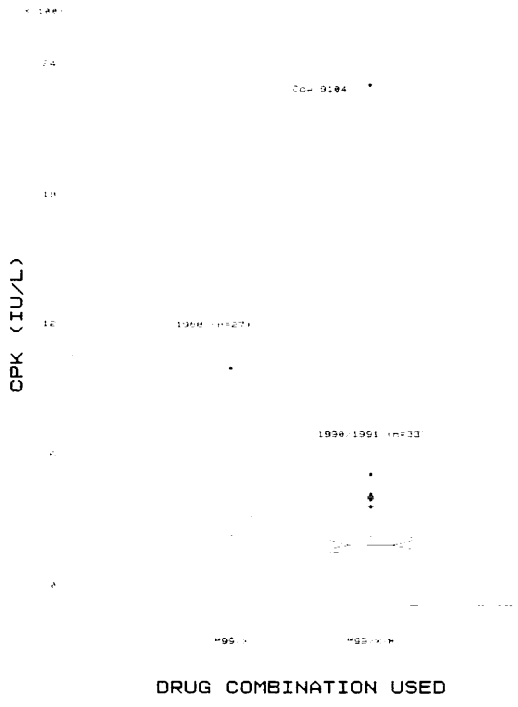

Figure 4. Comparison of relative data distributions of CPK values (IU/L) measured from blood samples collected soon after immobilization of black rhinoceroses with either M99/xylazine/hyaluronidase (combined 1990/1991 data) or M99/xylazine alone (1988).

often be quietly ambushed. After being darted they tend to be aggressive and run, which may explain the higher cortisol levels in these two animals. Removal of these animals from the nonstressed group with subsequent comparison between the 1988 and 1990 drug combinations revealed a significant difference in serum cortisol levels (1.67 ± 0.1 μg/dl and 1.15 ± 0.2 μg/dl; $P < 0.02$). Cortisol values (2.48 ± 0.17 μg/dl) for the 1991 immobilizations were higher than those for the 1990 group (1.67 ± 0.24 μg/dl) and similar to those of the 1988 group (2.12 ± 0.14 μg/dl) (Table 1), even though induction times for the 1991 group were significantly lower than those in either 1990 or 1988. As in 1990, the 1991 calves had higher cortisol levels than adults (3.13 μg/dl vs. 2.37 μg/dl).

Comparisons of serum cortisol between sampling periods 1 and 2 for the three capture years revealed significant increases, indicating that although rapid induction times may reduce the overall stress effect, with reduced body temperatures and muscle exertion, the combination of immobilization, prolonged recumbency, and sled transport is not without stressful effects. Evaluation of cortisol levels prior to reversal at the holding bomas (sampling period 2) for those rhinoceroses that were walked out of difficult terrain using nalorphine revealed higher cortisol levels when compared with those of animals that were loaded on the sled at the site of recumbency (4.4 ± 0.4 μg/dl, $n = 7$, vs. 3.5 ± 0.33 μg/dl, $n = 11$; $P < 0.1$). This increase indicates that walking black rhinoceroses from the immobilization site to the transport sled under partial M99 sedation elicits an apparently greater stress response than direct sled loading. However, walking some of the animals between 0.5 and 1 km was necessary because of the rough terrain, and the only alternative would have been release at the immobilization site.

Glucose values were significantly lower in the M99/xylazine/hyaluronidase group, indicating reduced stress (Table 1). Significantly lower CPK, AST, and LDH values at capture compared with the M99/xylazine group indicate less muscle damage[7] (Figs. 4, 5). In particular, LDH was markedly reduced in 1991 (Fig. 5), suggesting that LDH is a sensitive indicator of muscle damage in the black rhinoceros. Values for CPK, LDH, and glucose in 1990 and 1991 (Table 1) are below baseline values previously reported.[8] In the 1991 group of animals, one black rhinoceros cow skewed the data, with a CPK value at capture of 2,302 IU/L. The presence of severe moist inguinal dermatitis and possible myositis in this animal may explain the higher value. This animal had a CPK level of 2,326 IU/L at sampling period 2, a minimal increase of 24 IU/L after an immobilization period of 190 min.

Animals in the M99/xylazine/hyaluronidase groups were under M99 sedation con-

siderably longer than the M99/xylazine group, and total transport times were longer (Table 1). Despite the longer anesthetic and transport times and the more rugged terrain, serum glucose, CPK, AST, and LDH levels were significantly lower when measured prior to opioid reversal (sampling period 2)[7] (Table 2), indicating reduced stress and tissue damage as a result of rapid immobilization. Rapid immobilization appears to be more important in reducing stress than does reduction in the duration of anesthesia and transport times.[6]

Creatine phosphokinase values for sampling period 2 were skewed by high values for the four rhinoceroses who were immobilized for >8 hr. The CPK values for two adult cows in this group were 945 and 1,088 IU/L. The significantly lower CPK values (Table 2) for the M99/xylazine/hyaluronidase groups exclude these higher values.

The addition of long-acting neuroleptics to the management of recently captured black rhinoceroses has significantly reduced the adaptation period and the risk of self-inflicted trauma.[6,7] These long-acting neuroleptics probably did not affect the blood results from sampling period 2, because the drugs were either administered at the capture site while the animals were still anesthetized or at reversal after the blood sample was taken.

Rapid induction of anesthesia is achieved with hyaluronidase and M99, provided placement of the projectile dart is correct. Animals inadvertently darted in the chest or subcutaneously took longer to show signs of anesthesia and to become recumbent. Black rhinoceroses appear to tolerate extended anesthesia well; in 1991, two cow/calf combinations remained immobilized, because of logistics, for >8 hr. All four animals required walking between 0.5 and 1.5 km to the recovery vehicle, and despite extended anesthesia and transport, all underwent smooth, uncomplicated reversal. One adult cow was simultaneously darted twice (total dose M99 = 7 mg) in the 1991 capture

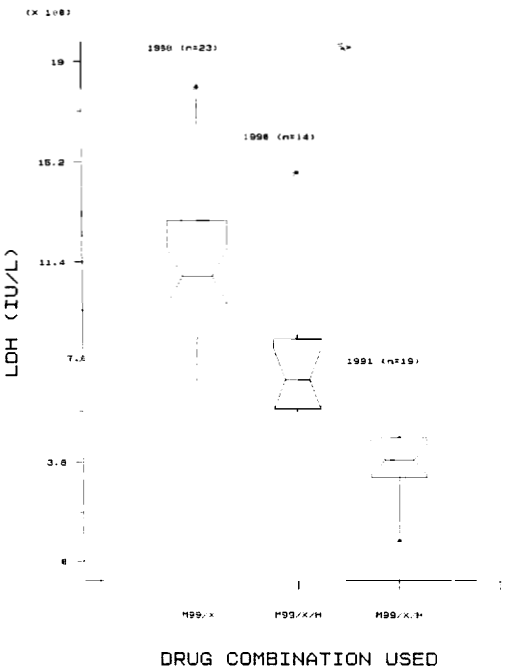

Figure 5. Comparison of relative data distributions of LDH values (IU/L) measured from blood samples collected soon after immobilization of black rhinoceroses with either M99/xylazine/hyaluronidase (1990 and 1991 data) or M99/xylazine alone (1988).

and became recumbent in 2 min. Despite the high M99 dose, she was very stable at capture (respiratory rate = 4 breaths/min; pulse rate = 68 beats/min) and responded well to doxapram and nalorphine.

The reduced induction times in 1990 compared with 1988 reflect modification of drug combinations and doses, and further reductions between 1990 and 1991 (Fig. 1), using the same drug combinations and doses, indicate the value of experienced capture teams. This experience resulted in more careful approaches to ground darting and better placement of darts.

The capture and translocation of black rhinoceroses in Zimbabwe has not been without problems.[6,7] Although identification of specific stressors contributing to mortality[8] remains obscure, management in 1990 and 1991 has concentrated on stress reduction at all stages for this endangered

species. The addition of hyaluronidase to immobilizing drug combinations, which increases permeability within the intercellular matrix of connective tissue[1] and therefore increases the rate of absorption, and the use of higher M99 doses has met with success in Namibia.[9] This protocol in Zimbabwe has also been successful in reducing capture-related stress.

An on-going adaptive management program includes the analysis of data following capture and boma confinement and during and following translocation in an effort to determine what, if any, management changes may further reduce stress and improve long-term survival of the black rhinoceros in Zimbabwe.

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