CHEMICAL IMMOBILIZATION OF FREE-RANGING BLACK RHINOCEROS (*DICEROS BICORNIS*) USING COMBINATIONS OF ETORPHINE (M99), FENTANYL, AND XYLAZINE

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Abstract: Fifty-two free-ranging black rhinoceroses (*Diceros bicornis*) of various ages were immobilized. Eleven rhinoceroses were darted on foot and 41 were darted from a helicopter. Twentyeight animals were immobilized using 3 mg etorphine and 100 mg xylazine per animal. Twentyfour animals were immobilized using a mean dose per animal ($\bar{x} \pm SE$) of 1.8 ± 0.13 mg etorphine and 30.9 ± 1.1 mg of fentanyl combined with a standard dose of 100 mg xylazine. Induction time for both of the etorphine combinations (n = 52) was 20.0 ± 2.5 min (median, 13.5 min), with no significant difference in induction time between the two combinations. The mean duration of immobilization was 184 ± 10.6 min. The mean reversal time following the administration of a narcotic antagonist was 3.0 ± 0.2 min (median, 2.5 min). Individual animals were placed into an outcome classification at capture: 29 were classified as normal, 23 as stressed. Of the rhinoceroses darted from a helicopter, 19 were classified as stressed, and of those animals darted on foot, four were classified as stressed. No direct capture-related fatalities occurred.

Key words: Black rhinoceros, Diceros bicornis, immobilization, etorphine, fentanyl, xylazine, azaperone, perphenazine enanthate, diprenorphine, naltrexone, stress.

INTRODUCTION

The black rhinoceros (*Diceros bicornis*) is threatened with extinction because of poaching and habitat destruction in Africa, with less than 4,000 black rhinoceroses remaining.⁷ Zimbabwe has <2,000 animals of which the Zambezi valley (16°00'S, 29°30'E) has a current estimated population of 600. This population has been severely reduced by poaching, and these poaching practices have prompted the Zimbabwe wildlife authorities to relocate more than 300 rhinoceroses since 1985 from the lower Zambezi valley to more secure areas elsewhere in Zimbabwe.

Early work on chemical capture of rhinoceros involved the black rhinoceros,¹² although more extensive work has since been carried out on the white rhinoceros (Ceratotherium simum).^{11,18,25,26} Several reports from the 1960's and 1970's^{4,5,10,14-17,19-21,23} concern capture and relocation of black rhinoceroses. In 1984, Flamand et al.⁸ made significant capture and management recommendations. Booth and Coetzee² described a series of capture operations in Zimbabwe prior to 1988. Active management of free-ranging black rhinoceroses at present is restricted to remaining populations in Kenya, South Africa, Namibia, and Zimbabwe. This paper presents detailed information on the chemical capture and transport of 52 black rhinoceroses from the lower Zambezi valley (n = 50) and Midlands area (n = 2) of Zimbabwe in 1988, using combinations of etorphine (M99), fentanyl, and xylazine.

MATERIALS AND METHODS

Fifty black rhinoceroses were captured and relocated from Mana Pools National Park and the Urungwe, Sapi, and Chewore Safari areas to other areas in Zimbabwe. In addition, two animals that had been captured and translocated to private land in

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1987, and had subsequently escaped, were recaptured and returned in May 1988.

All 52 rhinoceroses in this study were chemically immobilized in the early morning or late afternoon with a dart gun and projectile syringes. The animals were first located in the valley by a fixed-wing aircraft and darted by a ground team (n = 11) or were darted from a helicopter (n = 41). All the rhinoceroses were darted with a mixture of etorphine (M99, 4.9 mg/ml, Rickett and Coleman Pharmaceuticals [Pty], Ltd., Suidkusweg, 1474 So. Coast Road, Mobeni 4092, South Africa) and/or fentanyl (Sublimaze, 40 mg/ml, Ethnor [Pty], Ltd., Halfway House, South Africa), and xylazine (Rompun, 100 mg/ml, Bayer, Leverkusen, Germany). The narcotics were reversed using diprenorphine (M50-50, 12 mg/ml, Rickett and Coleman Pharmaceuticals) and/or naltrexone (50 mg/ml, Wildlife Laboratories Inc., Fort Collins, Colorado 80525, USA). All the drugs were stored away from heat and light at base camp. Because of the difficulty in accurately estimating body weights of black rhinoceroses in the field, a standard dose of 3 mg of etorphine and 100 mg of xylazine (M99/X) was used in 28 animals. Twenty-four rhinoceroses were immobilized with a combination of etorphine (1.8 \pm 0.13 mg), fentanyl (30.9 \pm 1.1 mg), and xylazine (100 mg) (M99/F/X). The addition of fentanyl was necessary because of a shortage of etorphine and allowed the reduction in the etorphine dosage.

Syringes were either loaded the night before or early in the morning of the day of capture. Most projectile syringes used were specially manufactured for rhino capture in Zimbabwe. These held 3 ml and had a 65- \times 0.5-mm reinforced collared needle and were based on the Palmer capture darts with the following modifications: more robust aluminum tubes to withstand impact pressures, feathered tailpieces (35 mm) with 'O' rings, reinforced nose sections, and needles constructed from steel injection tubing with four side porting holes and a closed end. The dart rifle used was a modified 20-gauge shotgun with open sights. Powder charges were used to propel the dart. Several rhinoceroses caught towards the end of the capture exercise were darted using a Palmer long-range rifle and Cap-chur darts (3 ml and 46- \times 0.5-mm collared needles) (Palmer Chemical and Equipment Co., Inc., Box 867, Douglasville, Georgia 30134, USA). The majority of the rhinoceroses were darted in the caudal thigh or dorsal thigh/rump area, but occasionally the shoulder was used; five animals were inadvertently darted in the abdomen, chest, or head, all fortunately without any complications.

After the animal was darted, the aircraft followed the animal and directed the ground team to it after signs of induction were apparent. If signs were not seen in approximately 10-15 min, the animal was reevaluated from the aircraft and redarted if appropriate. Once induction became evident, the ground crew, in four-wheel drive vehicles, was directed by radio from the aircraft to the animal. After the rhinoceros became immobile or sufficiently incoordinated, a team with ropes approached and secured the animal in lateral recumbency. The animal's eyes were covered, a clinical examination was performed, and physiological data, body measurements, blood samples, and other biological data were collected. The animal was rolled onto a wooden sled and firmly secured with ropes. Padding was placed under the head and legs. The sled was then winched onto the back of a four-wheel drive truck with a tipping tray and the animal was transported to the holding boma.

Individual rhinoceroses were classified into an outcome category at capture. This was based on evaluation of the animal prior to darting, during induction, and on a clinical examination when the animal was immobile. Those animals experiencing rapid induction without complications were placed in the normal category. There were three stress classifications: mild stress included those animals experiencing some agitation and excitement during darting and an extended induction period; moderately stressed animals experienced marked agitation, a prolonged chase prior to darting, and a prolonged induction period; severely stressed animals were markedly compromised with significant struggling, sweating, and signs of exhaustion, including openmouthed breathing, and immobilization was generally poor.

Many of the rhinoceroses were captured 150-200 km from base camp and required additional etorphine (1 mg i.v.) prior to or during transport to the bomas. At base camp, the sled was pulled into a recovery boma. the restraining ropes were removed (except those tied around the lower legs), and the animal was rolled off the sled and maintained in lateral recumbency. Once the animal was secure and lying quietly, an ear tag was placed and further physiological and biological data collected. Ages were estimated using standard toothwear procedures.^{6,13} Leg ropes were then removed and the narcotic antagonist given. Diprenorphine was administered at a standard dose rate of 12 mg (6 mg i.v. and 6 mg i.m., n =50). Naltrexone was used on occasion (25 mg i.m., n = 2) in combination with diprenorphine to reverse the effects of the etorphine/fentanyl mixture. Several very aggressive individuals were also given a longacting tranquilizer, perphenazine enanthate (Trilafon, Sherag [Pty], Ltd., 54 Electron Avenue, Isando, Transvaal, South Africa) to produce calming and prevent self-inflicted trauma.

Following various periods of boma confinement, rhinoceroses were crated individually, and the crates were loaded onto a flatbed truck for translocation. Most subadult and adult rhinoceroses received sedation prior to crating. Animals were darted in the neck or shoulder area with a projectile dart (Pneu-Dart, Inc., P. O. Box 1415, Williamsport, Pennsylvania 11703, USA) containing an appropriate drug. Following this, the animals were left undisturbed for 20–30 min prior to crating. Various combinations of etorphine (0.5–1 mg) with xylazine (18–75 mg) or azaperone (30–200 mg) (Stresnil, Janssen Pharmaceutica, B. V. Goirle, The Netherlands) were used to produce mild degrees of sedation. Sedated rhinoceroses were pulled into the crates with head-ropes if they did not enter them voluntarily when the crate doors were opened. At this stage, an acaricide pour-on solution (Drastic Deadline, Flumethrin 1% pour-on, Agricura [Bayer], P.O. Box 2742, Harare, Zimbabwe) was applied along the back.

Data were analyzed using a statistical graphics program (StatGraphics, Statistical Graphics Corp., Rockville, Maryland 20850, USA). Initially, exploratory data analysis was performed to evaluate distribution patterns, create data sets, and cross-tabulate data. Afterwards, specific statistical tests³ were applied, including one-way analysis of variance (ANOVA) (drug combination versus induction times, distance moved, and darting situation versus induction times), two-sample analysis using Student's *t*-test (comparison of temperature, respiration, and pulse with drug combination used; comparison of temperature, respiration, and pulse with number of darts used), and the chi-square test for independence (outcome classification versus darting situation, number of darts used, and drug combination used; muscle relaxation versus drug combination used; tractability versus drug combination used). Because the "raw" data for induction times and distance moved were distributed log-normally, logarithmic transformation allowed a nearer approximation to a Gaussian distribution for ANOVA. Means are reported with standard errors (SE).

Total induction time refers to the time from dart impact to when the animal became immobile, including both recumbency and standing sedation. Tractability refers to the degree of narcotic sedation and, therefore, the ability of the capture team to safely handle the rhino without further sedation. The time to first signs of reversal refers to the time from administration of the narcotic antagonist to the first movement of the ears and eyes, lifting of the head, and/or struggling, and total time of reversal refers to the time from administration of the antagonist to the time when the animal stood. Weights of all the rhinoceroses captured were estimated retrospectively from body measurements.⁹

RESULTS

The ratio of male : female rhinoceroses captured was 1.08:1.0. Of the 52 rhinoceroses captured, 42 were adults, five were subadults, and five were calves (<3 yr old). Estimated weights ranged from 158 to 1,336 kg. Estimated mean body weight was 894 kg (range, 666–1,175 kg) for adult males and 861 kg (range, 587–1,336 kg) for females.

Table 1 and Figures 1–4 show the results of the immobilizations. The mean induction time ($\bar{x} \pm SE$) for the total number of animals immobilized (n = 52) was 20.5 \pm 2.5 min. However, the median time to immobilization (13.5 min) probably reflects the true induction time more realistically because some animals were lost from sight during induction and had to be tracked on the ground, prolonging the recorded induction time. Others required multiple darts for adequate immobilization, and these animals had prolonged induction times. There was no statistically significant difference in the induction times between the two drug combinations (M99/xylazine, $\bar{x} = 24$ min, median = 16 min; M99/fentanyl/xylazine, $\bar{x} = 16 \text{ min}, \text{ median} = 13 \text{ min}; P < 0.08)$ or induction times between those animals darted on foot and those darted from the helicopter (P < 0.69). On average, rhinoceroses moved 2.4 \pm 0.31 km following darting. There was a significant difference in distance moved between the two drug combinations (M99/X, $\bar{x} = 2.9 \pm 0.48$ km, n = 25; M99/F/X, $\bar{x} = 1.8 \pm 0.36$ km, n =21; P < 0.05), possibly reflecting a more rapid development of incoordination and narcosis with the M99/F/X. Induction was characterized by drooping of the ears, failure of the animal to move around objects, stumbling, and increasing incoordination.

Many animals became immobile simply because the individual was unable to negotiate an obstacle such as a tree, dry river bed, or steep bank.

Rhinoceroses were recumbent for an average of 182 min, and this reflected the distance the capture team had to move from base camp to locate and capture animals; the average transport time was 100 min, and considerable additional time was involved in clearing a track to each immobilized animal. The degree of muscle relaxation under both drug combinations varied, with 32 of 52 (62%) rhinoceroses having adequate muscle relaxation and 20 of 52 (38%) poor muscle relaxation. Of the total number of rhinoceroses captured, 32 (32/52, 62%) were considered tractable enough to be rolled onto the sled without further drug dosing, 13 (13/ 52, 25%) were considered poorly sedated but with physical restraint (ropes) could be manipulated, and seven (7/52, 13%) needed additional narcotic for handling and rolling onto the sled. Table 2 shows the degrees of muscle relaxation and tractability within drug combinations. Animals immobilized with M99/F/X had better muscle relaxation than those with the M99/X combination (0.05 > P > 0.025). There was no apparent association between tractability and drug combination (0.1 > P > 0.05), but it appeared that the M99/F/X combination produced better overall sedation (Table 2).

Physiological parameters were monitored in the majority of rhinoceroses (Table 1). There were no significant differences in temperature, pulse, or respiration between the different drug combinations. There was a significant difference in respiration (P <0.005) between those rhinoceroses immobilized with a single dart and those in which multiple darts were used. No significant differences were seen in temperature and pulse. Both drug combinations produced respiratory depression in certain animals (low = 4-6 respirations/min), and this most often occurred in subadult animals, probably reflecting a slight overdose of narcotic. No individuals required reversal because of

Measurement	n	Mean	Median	SEª	Range
Induction time (min)	52	20.5	13.5	2.5	2-110
	45 [⊾]	15	13	1.3	2-38
Distance moved during induction (km)	46	2.4	2.0	0.3	0.25-9
Total down time (min)	52	182	157	11	61-360
Total transport time (min) ^c	52	100	98	7	22-231
Time to first signs of reversal (sec)	34	96	90	8	35-300
Time to standing (sec)	52	191	148	17.4	90780
	51ª	180	145	13	90-480
Temperature (C ^o)	52	38.75	38.75	0.12	36.5-41.2
Respiration (bpm)	50	11	10.5	0.84	4-35
Pulse (bpm)	42	80	72	3.8	42-160

Table 1. Values for induction time, distance moved following darting, time to first signs and complete reversal following administration of a narcotic antagonist, total down time, total transport time, and physiological values in black rhinoceroses chemically immobilized in Zimbabwe.

^a SE = standard error.

^b Seven animals were excluded because of prolonged induction.

^c Total transport time represents the time taken to transport an individual rhino from the darting location to holding bomas.

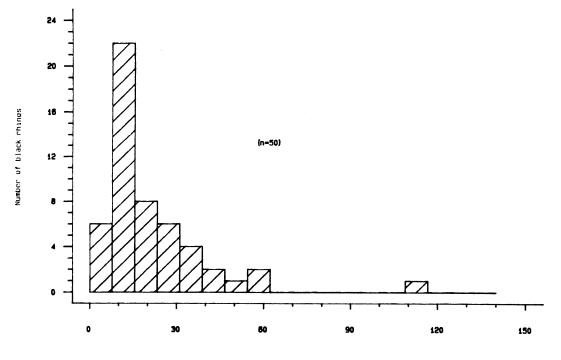
^d One animal was excluded because of a prolonged reversal associated with i.m. injection of the narcotic antagonist.

cardiovascular or respiratory system complications.

nist was 1.6 ± 0.13 min, and total time to

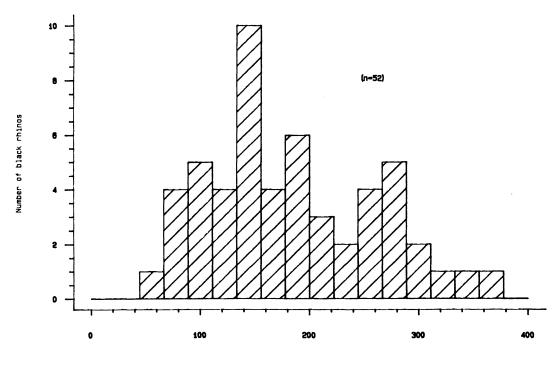
standing and ambulatory was 3.18 ± 0.29 min. One animal received 12 mg dipren-The time to first signs of reversal followorphine i.m. and had an extended recovery ing administration of the narcotic antagotime (13 min).

Of the 52 animals immobilized by dart



Induction time (mins)

Figure 1. Distribution of induction times for black rhinoceroses immobilized with mixtures of etorphine, fentanyl, and xylazine.



Total duration of anaesthesia (mins)

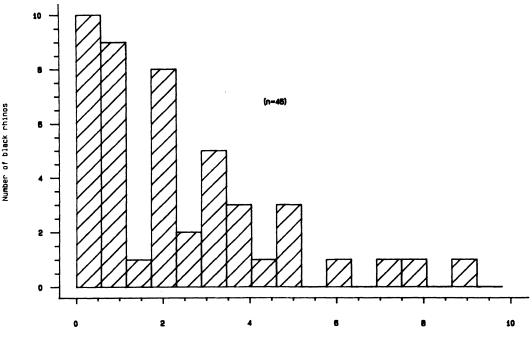
Figure 2. Distribution of total anesthetic times for black rhinoceroses immobilized with mixtures of etorphine, fentanyl, and xylazine. Time measured from capture to reversal in recovery boma.

gun, 35 required a single dart of either drug combination to achieve adequate sedation, 13 required more than one dart, and four animals required three or more darts before becoming immobile. Of those rhinoceroses approached on foot (n = 11), nine were successfully immobilized by a single dart, a success rate of 81%; of those rhinoceroses approached by helicopter (n = 41), 26 were successfully immobilized by a single dart, a success rate of 63%. Of the animals requiring additional doses of narcotic, 19 animals received two doses of narcotic, eight received three doses, and two received four doses. None of the subadults captured (n =5) required additional doses, even with long transport times, probably reflecting deep sedation from a standardized etorphine dose (2-3 mg) administered to all rhinoceroses regardless of size.

Twenty-three rhinoceroses were classified as stressed (44.2%), of which seven (13.5%) were considered mildly stressed, 11 (21.2%)

moderately stressed, and five (9.6%) as severely stressed. There were no direct capture-related mortalities. There was no statistical association between outcome classification and immobilization by foot or helicopter or with the different drug combinations, but there was a significant association between outcome classification and the use of one or more darts (0.05 > P >(0.025); those animals requiring more than one dart to achieve adequate sedation were more likely to be classified as stressed. Twelve out of 35 rhinoceroses who were immobilized with a single dart were considered stressed (34%), eight out of 13 rhinoceroses who required two darts were considered stressed (62%), and three out of four rhinos who required three darts were considered stressed (75%).

Following a period of boma confinement and habituation, rhinoceroses were loaded into crates for translocation. Most rhinoceroses were sedated prior to crating and fol-



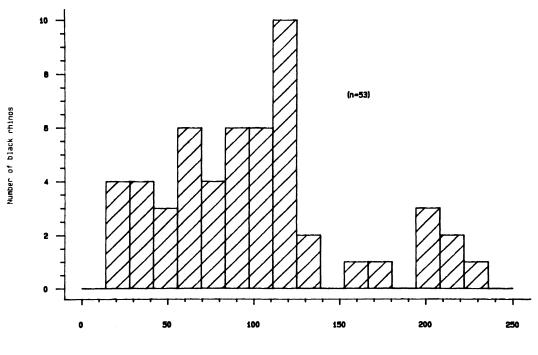
Distance moved (km)

Figure 3. Distribution of distances moved by black rhinoceroses from the time of darting until recumbency or immobilization.

lowing sedation often walked quietly into the crates. Some required roping over the front horn and behind the lower jaw and minimal force to guide them into the crates. Ultimately, this loading method was highly successful in reducing stress-related problems. The dose of narcotic, in most cases, produced standing sedation in the crates that lasted for 2-3 hr. The minimal effective dose of etorphine was approximately 0.5 mg/animal. When the animals arrived at their destination, they were usually active and aggressive. The use of xylazine (100 mg) or azaperone (200 mg) alone was not adequate in calming excited animals for crating, although these drugs were effective at higher doses (150-200 mg xylazine and 200-400 mg azaperone) for calming some individuals.

DISCUSSION

Work performed in the 1960's on chemical immobilization of both white and black rhinoceroses, using narcotics and tranquilizers, showed the potential for the use of chemical restraint methods for management and conservation purposes.^{11,12} Several reports describe the capture of black rhinoceroses by darting on foot, from a vehicle, and/or helicopter.^{4,10,15,19,21} Darting of individual rhinoceroses on foot was considered difficult and time-consuming;²¹ those animals darted on foot in our study also presented difficulties because of thick vegetation, variable wind conditions, and the presence of red-billed oxpeckers (Buphagus erythrorhynchus) who provided early warning to the rhinoceros. Other researchers have successfully immobilized rhinoceroses in the Zambezi valley using ground methods.² The doses of narcotic used in this study were higher than those used by Booth and Coetzee² who reported on the capture of black rhinoceroses from the Zambezi valley in 1984-1985. Rhinoceroses have been successfully immobilized in Namibia with doses



Total transport time (mins)

Figure 4. Distribution of total transport times for immobilized black rhinoceros from capture sites to holding bomas.

of M99 as high as 4.5–5.5 mg for adults males and 4–4.5 mg for adult females, combined with azaperone. The addition of hyalase (Hyaluronidase, Sigma Chemicals, Poole, Dorset, England) to the projectile sy-

Table 2. Number of animals categorized by drug combinations, degree of muscle relaxation, and tractability in a study of black rhinoceroses chemically immobilized in Zimbabwe.

	Drug combination			
Measurement	M99/ xylazine (n = 28)	M99/ fentanyl/ xylazine (n = 24)		
Muscle relaxation				
Good	14 (50%)	18 (75%)		
Poor	13 (46%)	5 (21%)		
Very poor	1 (4%)	1 (4.1%)		
Tractability				
Good	14 (50%)	18 (75%)		
Poor but still handleable Poor, additional drug	8 (29%)	5 (21%)		
dosing required	6 (21%)	1 (4%)		

ringe (4,500 units) resulted in induction times of 3–6 min.²⁴ High doses of narcotics (>4.0 mg) would need to be used with caution in the Zambezi valley because of the thick vegetation and the danger of prolonged recumbency with respiratory depression if the animals were not located quickly.

Flamand et al.⁸ do not recommend the use of a sled for transport, as was used in this study, because of the risk of radial paralysis with prolonged lateral recumbency. Its use, however, has been reported by others,^{10,20} and none of the animals in this study experienced permanent detrimental effects that were obviously due to sledding and prolonged lateral recumbency. Despite an average transport time on a sled of 100 min (range, 22–231 min), only one animal in this study showed evidence of lameness that persisted for a few days and then resolved completely. However, the management technique of crating the animals at the capture site has many advantages, and further work evaluating stress and trauma related to sled transport in the Zambezi operations may provide data to support crating.

This is the first report of immobilization using combinations of narcotics with xylazine for the black rhinoceros. The effect of 100 mg of xylazine given to an animal was difficult to determine, and none of the animals showed real evidence of xylazine sedation following narcotic reversal. However, this was often difficult to evaluate because of the typically aggressive behavior that followed narcotic reversal. Those animals immobilized with the M99/F/X mixture showed various degrees of sedation following narcotic reversal, but this may have been a result of incomplete reversal of fentanyl with diprenorphine⁸ and not of any sedative effect of xylazine. It is possible that the xylazine smoothed out the induction period. but a more controlled study would be necessary to confirm this. Flamand et al.8 believe there is little advantage in adding a tranquilizer (azaperone) to the narcotic mixture and that etorphine and fentanyl together are superior to etorphine alone. Our results support this, as the mixture of M99/ F/X produced a more rapid and smoother induction and better muscle relaxation than did M99/X. In addition, these rhinos moved less following darting and were less stressed. However, others have successfully immobilized rhinoceroses with etorphine alone.²

The mean induction time of 20 min in this study reflects the effects on the data set of prolonged induction of several animals (range, 34–110 min). The median induction time of 13.5 min compares favorably with other recent reports in the literature.^{2,10,15,20} Prolonged inductions occurred in several animals because the thick bush made it difficult for the spotter plane to keep the fleeing animal in visual contact, making tracking necessary. It was not possible with these animals to establish true induction times as immobilization was recorded as complete when the animal was located. In these cases, induction time was recorded as the time from darting to location of the animal. Rhinoceroses that were darted in heavy muscle masses on average showed a smoother and more rapid induction compared with those darted in the abdomen, chest, or head. For example, one rhinoceros darted behind the eye had an induction time of 30 min, and another darted in the abdomen had an induction time of 18 min. One animal darted in the back muscles was completely immobile at 2 min, probably reflecting injection of the drug through or into a blood vessel. The use of hyalase in future capture operations may reduce the induction time to <5 min.

Narcotic reversal was effectively achieved with diprenorphine (M50-50). It was rapid and uncomplicated in the majority of rhinoceroses (Table 1). Narcotic recycling was not a problem in this study. Some of the animals in this study that were immobilized with the M99/F/X combination and reversed with diprenorphine alone continued to show narcotic signs such as unsteadiness and sweating for a few hours after reversal. These signs were probably a result of incomplete reversal of the fentanyl component. Naltrexone, an experimental narcotic antagonist, was used in combination with diprenorphine to reverse M99/F on occasion, and reversal appeared complete.

The period of boma confinement for the 52 black rhinoceroses captured varied from 24 hr to 19 days. The majority of the rhinoceroses settled into captivity without complications, but several were aggressive after recovery and some lost front horns secondary to self-induced trauma. A longacting tranquilizer (LAT), perphenazine enanthate (Trilafon), was administered at a dose rate of 200-400 mg/animal i.m. in an attempt to calm some of the more aggressive animals. Although it was difficult to evaluate objectively, there is a suggestion that these individuals were also calmer after translocation and during further boma confinement. Further investigations as to the efficacy of LAT's in the black rhinoceros are being carried out, and the results so far are promising. Keep et al.¹⁹ report on the use of perphenazine enanthate in three black rhinoceroses but only mention the inclusion of this drug to potentiate the narcotic effect of etorphine.

The success of a capture operation should not be judged solely on the numbers captured or mortality at capture, but rather on the long-term survival of the relocated animals, with successful breeding the major criterium of success.^{1,22} The capture and relocation of 52 black rhinoceroses from the lower Zambezi valley and Midlands in July 1988 was successfully carried out without any direct capture-related mortalities. However, investigations into long-term survival of these translocated animals have revealed an indirect mortality rate of 17% (9/52) from 1 wk to 2 mo postcapture, and previous capture operations in Zimbabwe have had similar indirect mortality rates (13% overall since 1985). Prior to 1988, the monitoring of translocated rhinoceroses was inadequate with little information on survival status. Investigations carried out in 1988–1989 revealed unacceptably high mortalities related to translocation. The response of the black rhinoceros to acute and chronic stressors and the causes of mortalities are currently being summarized.

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