

Training as a Tool for Routine Collection of Blood from a Black Rhinoceros (*Diceros bicornis*)

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Introduction

The black rhinoceros (*Diceros bicornis*) is one of the world's most critically endangered mammals. Black rhino numbers have dropped from an estimated 60,000 in 1960 to a current level of under 2500 (Maruska, 1993). The captive North American population as of 1 July 1993 was 98 individuals (Maruska, 1993). As wild populations decline, successful management of captive populations becomes increasingly important. Captive breeding of black rhinos has been disappointing and is not adequate to maintain the genetic diversity of the captive population (Maruska, 1993). The 1992-93 American Zoo and Aquarium Association (AZA) Rhino Taxon Advisory Group report emphasizes the need to intensify research on reproduction, nutrition, and health in these animals (Reece, 1993).

Basic information on reproductive cycles of black rhino females is critically needed. Equally important is the development of safe and consistent methods to monitor these reproductive cycles in captive rhinos. Reproductive physiologists have developed assays for saliva, urine, and feces with some success, as possible safe methods to monitor hormonal changes (Czekala, 1989; Berkeley, pers. comm.). Blood, however, remains the most direct medium for examining ovarian function (Czekala, 1989), and the development of non-invasive procedures such as fecal and urine steroid hormone analysis should be validated by correlation with serum hormone concentrations (Berkeley, pers. comm.). Blood hormone levels are measured less frequently due to the difficulty and risks of obtaining samples (Berkeley, pers. comm.). Techniques for safe routine blood collection in rhinos would facilitate not only reproductive research, but also nutrition studies, artificial insemination and veterinary work.

In 1991, animal keepers and researchers at Metro Washington Park Zoo (MWPZ), Portland, OR, became interested in following the onset of puberty in a young female black rhino. We planned to look simultaneously at behavioral and physiological measures of reproduction (behavioral observations four to five times each week and via serum progesterone levels in blood). Initially, it was intended to modify our existing restraint chute so that routine blood collection from an unanesthetized animal could be safely accomplished. It became apparent that a chute remodel was not possible given the restrictions of building design and budget. As an alternative to manual or chemical restraint of the female rhino, a program of training was initiated using an operant conditioning, positive reinforcement technique. Training offers the advantage of reduced stress to the animal (Bloomsmith, 1992; Laule, 1992; Reichard, 1992), and may allow for more reliable sample collection (Laule, 1992; Reichard, 1992). Additionally, we reduced the possible effects of stress or anesthetics on our blood samples and on the animal's behavior (Bloomsmith, 1992). The objective of this study was to train the female rhino to cooperate with keepers and to obtain weekly blood samples without the use of restraint or anesthesia.

Method

MWPZ maintains 1.1 black rhinoceros: "Miadi", the female born 4 November 1988 at the Brookfield Zoo in Chicago and "Pete", the male born 7 May 1988 at the Denver Zoo. The rhino holding units are in the same building as the hippo holding areas. There are three indoor holding areas for rhino: one 5.5m by 5.8m (18 ft. x 19 ft.); one 5.8m by 6.4m (19 ft. x 21 ft.); one 4.6m by 5.5m (15 ft. x 18 ft.); and one partially covered outdoor holding area 5.5m by 4.9m (18 ft. x 16 ft.). Each is connected by hydraulically controlled doors. Holding units are divided by concrete filled metal posts spaced approximately 20cm (8 in.) apart. The outdoor exhibit yard is 36.6m by 12.2m (120 ft. x 40 ft.) with a pool at one end. The rhinos are housed separately, in adjacent rooms at night and share the exhibit yard during the day. Training takes place along a specific section of posts in Miadi's holding area. No other interaction with the rhino takes place in this specific area.

Our goal was to train voluntary cooperation with blood collection without the use of a restraint device or anesthesia. This involved two separate training phases. First, training Miadi to come on command and stand parallel to the bars with her head steady during the procedure. Second, to desensitize Miadi to the equipment and handling necessary to draw blood. One keeper was designated as the primary trainer until the rhino reliably executed the behaviors required for successful blood collection.

The first step was to train her to come to a specific spot; essentially to "target" to the trainer's hand on command. The command was "Miadi, come". The word "good" was used as a bridge and apples were used as the reinforcement for the appropriate behavior. After she was responding consistently to the "come" command, a second trainer was added to increase her reliability by scratching her belly with a long handled scrub brush. This continuous reinforcement also encouraged her to stand parallel to the bars.

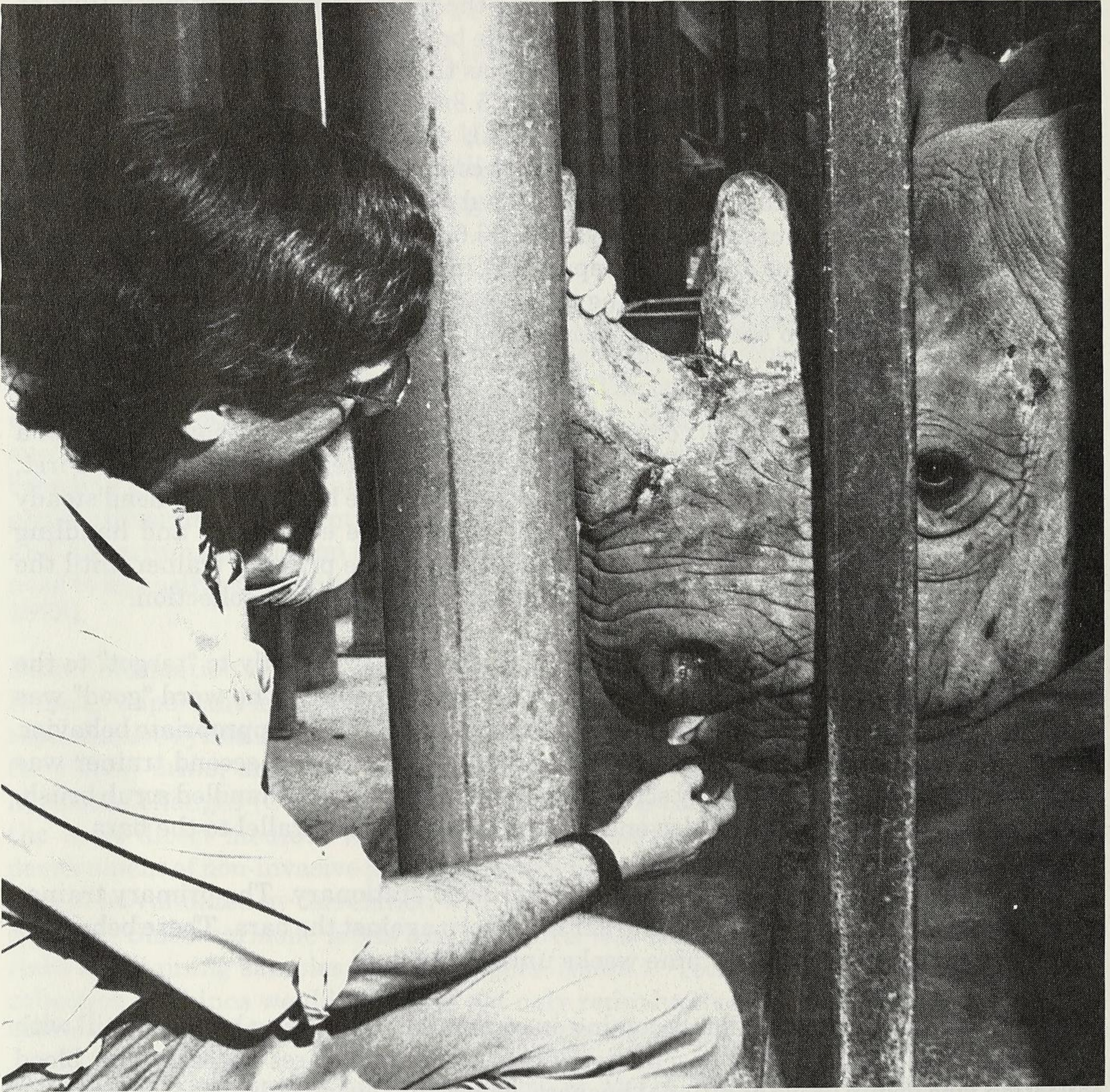
The next step was to train the rhino to hold her head stationary. The primary trainer used the command "stay" while holding her front horn against the bars. These behaviors were shaped for approximately nine weeks until consistent.

Once the female rhino was reliably coming on command and holding her head still with her body parallel to the bars, desensitizing her to the procedures necessary for blood collection was begun. Two additional keepers, who would eventually be performing the actual venipuncture, were then introduced to the process. Manipulation of the rhino's ears was begun by rubbing and pinching them. A tourniquet was draped over Miadi's ear, working gradually toward tying it around the ear. The rhino was then desensitized to the smell of an iodine based antiseptic and to having her ear scrubbed.

Concurrently, the rhino was introduced to the equipment used for drawing blood. A 21-gauge butterfly infusion set with a 12" tube on a 6cc syringe was used. The keepers let the tube and syringe hang near her eye and face until the rhino showed no reaction to the equipment. A blunted practice needle was pressed against the dorsal surface of the ear, gradually adding pressure until again the rhino exhibited no reaction. The desensitization process took about five weeks.

The two keepers who drew the blood had previous experience performing venipuncture. Procedures were established by the zoo's research veterinarian and a training session

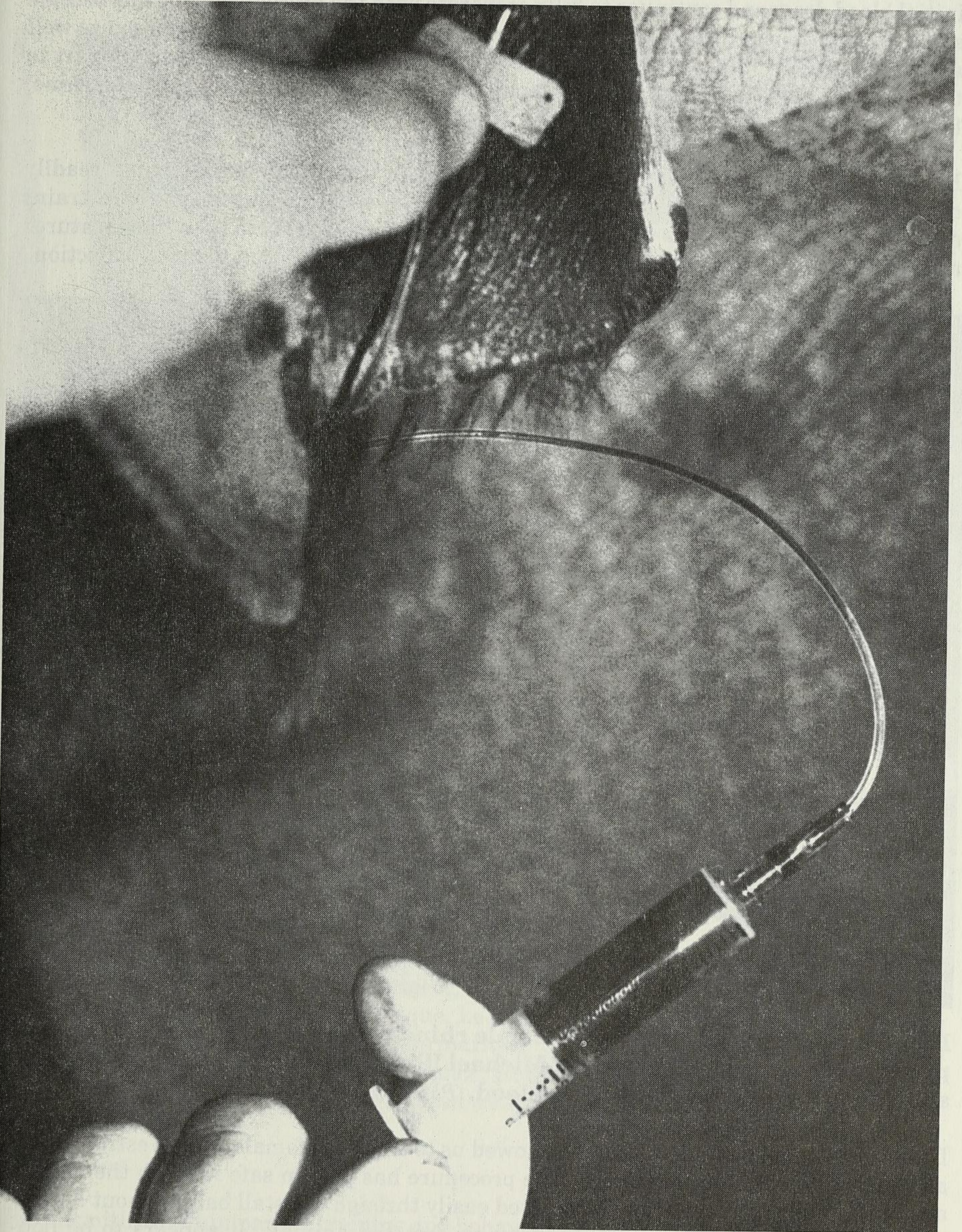
was done using pygmy goats to verify the correctness of the keeper/phlebotomist's techniques.



Keeper Michael Illig delivers a reinforcement to the rhino for holding her head stationary. (*Photo by Michael Durham*)

On days that blood was drawn, heat in the building was raised to approximately 85 ° F (29.4° C) for two hours prior to the procedure. This aided blood collection by dilating the veins of the ear and increasing blood flow. Signs were also posted outside the building warning that the blood draw was in progress to prevent unexpected interruptions that could startle the rhino or the phlebotomist. Blood was successfully drawn after a total of approximately five months of training. Weekly samples have been obtained from that time to the present with plans to continue indefinitely. Details from analysis of these blood samples are in preparation (Michel et. al., in prep). We have confirmed that this female is cycling but to date, is not pregnant. Low levels of progesterone correspond with reproductive behavior (Mellen et. al., in prep).

Each week approximately 5cc of blood are drawn. The procedure takes five to ten minutes. The sample is centrifuged and the serum is frozen. At six-month intervals the samples are analyzed for progesterone levels. Since November of 1994 weekly hematocrit testing has also been done.

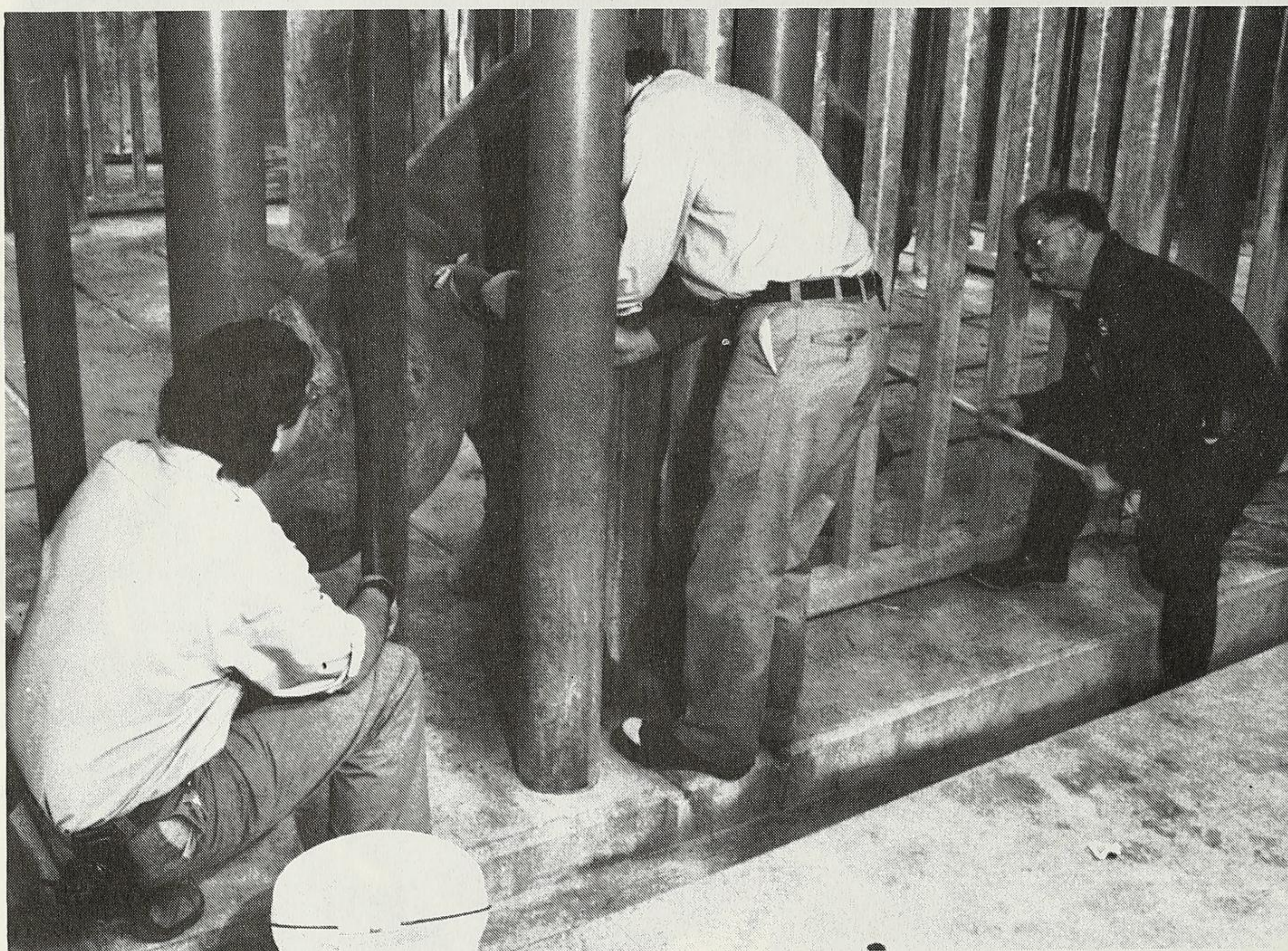


Blood is drawn from an ear vein using a 21-gauge butterfly infusion set with 12-inch tubing. Keeper David Illig draws blood from our female rhino Miadi.
(Photo by Michael Durham)

Discussion

The first blood draw from Miadi was performed on 15 May 1993, approximately five months after training was begun. The rhino exhibited almost no reaction during the initial blood draw and continues to fully cooperate with the procedure. She has become so comfortable with the procedure that she will tolerate observers and cameras as well as changes in personnel. It is felt that the success of this project may be due in part to the exclusive involvement of animal keepers, those who work routinely with the rhino, for training and blood collection.

Blood collection has become a routine procedure in which our female rhino readily cooperates. This procedure has been successful every week without the use of restraint or drugs at any time. This training regime has also allowed us to take temperatures and do simple physical exams and medical treatments, in addition to blood collection.



Blood has been drawn from our female rhino every week for two years using positive reinforcement training. Michael Illig, David Illig and Lee Jensen are shown in the process of drawing blood. (Photo by Michael Durham)

Data from these blood samples have allowed us to track the female rhino's estrous cycle and to monitor her for pregnancy. The procedure has proven safe for both the animal and staff. The rhino's ear can be accessed easily through the stall bars without danger of the phlebotomist becoming injured by an agitated animal. It appears to be non-stressful for the animal and after the initial training, requires little time. We have collected successfully on days when our female rhino has been in estrous and therefore in an excited state. In fact, the procedure may actually have a calming effect on her.

We feel that we will be able to get blood from her in the event of a medical emergency. After over a year and a half of weekly blood collection, it is felt that training is a safe and practical approach to blood sampling.



Keeper Lee Jensen demonstrates continuous positive reinforcement. This aids in aligning the rhino parallel to the bars of her enclosure. *(Photo by Michael Durham)*

Acknowledgments

The authors gratefully acknowledge animals keepers David Illig and Lee Jensen for their weekly participation in this project; animal keeper Diane Gould, for discovering the continuous reinforcement technique, i.e. the use of the scrub brush on the rhino's belly, essential to the procedure; Veterinarians Dr. Michael Schmidt and Dr. Mitch Finnegan, for their support and assistance; Research Coordinator Dr. Jill Mellen for her support; and E. V. Berkeley of Deaconess Research Institute, Billings, MT for her assistance.

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