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## ENDOCRINE AND METABOLIC RESPONSES OF THE COLLARED PECCARY (*TAYASSU TAJACU*) TO IMMOBILIZATION WITH KETAMINE HYDROCHLORIDE

Eric C. Hellgren,<sup>1,3</sup> Robert L. Lochmiller,<sup>1</sup> Max S. Amoss, Jr.,<sup>2</sup> and William E. Grant<sup>1</sup>

**ABSTRACT:** Serial physiological responses were examined for 150 min from captive collared peccaries during immobilization with ketamine hydrochloride. Rectal temperatures decreased significantly ( $P < 0.01$ ) during anesthesia. Serum concentrations of total proteins, albumin, cholesterol, alanine aminotransferase, and calcium declined significantly ( $P < 0.05$ ) during the first 45 min post-immobilization before stabilizing. Concentrations of lactate dehydrogenase and alkaline phosphatase in sera showed similar but nonsignificant ( $P > 0.05$ ) trends. Inorganic phosphorus and aspartate aminotransferase concentrations increased significantly ( $P < 0.05$ ) throughout the trial. Concentrations of serum glucose and glucocorticoid during the immobilization period were highly variable between individuals. Serum electrolytes, urea nitrogen, creatinine, gamma-glutamyl transferase and progesterone were not significantly ( $P > 0.05$ ) affected by immobilization. Elevations in serum testosterone were noted. Results indicated appropriate sampling times relative to immobilization for assay of particular serum biochemicals and steroid hormones during investigations of the physiology of the collared peccary.

### INTRODUCTION

Physiologic monitoring of the reproductive and nutritional status of free-ranging and captive wildlife is a rapidly expanding field. Use of potent, safe drugs for immobilization has provided a means for obtaining samples of blood and a variety of measurements with relative ease and safety. However, the physiologic environment is capable of reacting quickly to stressors and drugs. Acute capture-handling stress and pharmacological side-effects of drug administration can influence homeostatic mechanisms responsible for maintaining normal serum biochemical and hormone concentrations (Drevemo and Karstad, 1974; Wesson et al., 1979a, b; Gibson, 1980; Mautz et al., 1980). Wesson et al. (1979a) have discussed the need to describe effects of method of restraint

on serum analyses and to standardize handling and sampling techniques to properly interpret physiological data.

The objective of this study was to describe acute metabolic and hormonal changes to immobilization by ketamine hydrochloride in adult male and female collared peccaries. This method of restraint has been used previously in studies on wild peccary hematology (Lochmiller et al., 1985) and serum chemistry (Lochmiller and Grant, 1984).

### MATERIALS AND METHODS

Four adult collared peccaries (two males and two pregnant females) maintained on a commercial pelleted hog ration for at least 6 mo in a 900-m<sup>2</sup> outdoor enclosure at Texas A&M University were used in this experiment. During February 1984 animals were herded individually, with a minimum of excitement, into 2-m by 3-m pens and immobilized with an intramuscular injection (hindlimb) of ketamine hydrochloride (20 mg/kg) administered by blow-gun dart syringe (Lochmiller and Grant, 1983). Animals were placed in dorsal recumbency during the period of immobilization. Blood was collected by anterior vena cava venipuncture (Lochmiller et al., 1984). Rectal temperatures and blood samples (10 ml) were obtained at onset of immobilization (10-12 min post-injection) and at 15, 30, 45, 60, 90, 120, and 150

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min post-immobilization. Body weights were determined to the nearest 0.1 kg using a dial scale and ranged from 22.5 to 27.0 kg. Following the initial blood collection, a supplemental dose of ketamine was administered by hand injection to increase total dosage in all animals to 27.5 mg/kg. Blood samples were allowed to clot for 1 hr, centrifuged at 3,000 rpm for 15 min, and serum frozen at  $-20^{\circ}\text{C}$  for later analysis.

Serum samples were analyzed as a batch on a Technicon SMAC biochemical analyzer (Technicon Instruments Corp., Tarrytown, New York 10591, USA) for concentrations of total protein, albumin, urea nitrogen, creatinine, glucose, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), lactate dehydrogenase (LD), calcium, inorganic phosphorus, sodium, chloride, and potassium as previously described (Lochmiller and Grant, 1984). Concentrations of serum glucocorticoid were determined in duplicate 10- $\mu\text{l}$  aliquots without an extraction step using reagents supplied by Radioassay Systems Laboratory, Inc. (20770 Leapwood Avenue, Carson, California 90746, USA). Assay sensitivity was 0.5  $\mu\text{g}/\text{dl}$  and the intra-assay coefficient of variation was 13.2%. Progesterone (in female samples) was assayed in 100- $\mu\text{l}$  aliquots as described by Abraham et al. (1971) following extraction with 4 ml ethyl ether. Reagents and assay parameters are described in Hellgren et al. (1985). Sensitivity of the assay was 31.25 pg and intra-assay coefficient of variation was 4.3%. Testosterone (in male samples) was assayed in 50- $\mu\text{l}$  aliquots as described by Abraham et al. (1971) following extraction with 4 ml ethyl ether. Reagents and assay parameters are described in Hellgren (1984). Sensitivity of the assay was 3.25 pg and intra-assay coefficient of variation was 9.0%.

The General Linear Models procedure of the Statistical Analysis System was used in data analysis (Helwig and Council, 1979). Differences in serum metabolic and hormonal values between times post-immobilization were tested by a one-way analysis of variance for repeated measures. When the main effect was significant ( $P < 0.05$ ), differences between means were tested using Duncan's New Multiple Range Test.

## RESULTS AND DISCUSSION

### Rectal temperatures

Rectal temperatures dropped significantly ( $P < 0.01$ ) following immobilization from a mean of  $39.0 \pm 0.3$  (SE)  $^{\circ}\text{C}$  at the initial sample to  $37.1 \pm 0.5$   $^{\circ}\text{C}$  at 120

min post-immobilization (Fig. 1). Phelps (1971) reported that deep body temperature averaged 0.15  $^{\circ}\text{C}$  higher than rectal temperature, with diurnal fluctuations from 37.0 to 38.6  $^{\circ}\text{C}$  for a single telemetrically monitored male peccary. Initial body temperature was elevated due to excitement and slight enhancement of skeletal muscle tone produced by light ketamine anesthesia. As depth of ketamine anesthesia increased, muscle tone decreased and body temperature dropped. Rise in rectal temperature of female R48 at the end of the sampling period was attributed to early recovery from anesthesia. Ambient temperature ranged from 7–16  $^{\circ}\text{C}$  during sampling.

### Serum protein and nonprotein nitrogen

Concentrations of total serum protein and albumin decreased significantly ( $P < 0.01$ ) between 0 and 45 min post-immobilization, then increased to a constant concentration by 90 min post-immobilization (Fig. 1). Initial concentrations were greater than those at 150 min post-immobilization. High initial values and subsequent decline in serum protein and serum albumin are consistent with reports by Seal et al. (1972) and Wesson et al. (1979a) in white-tailed deer (*Odocoileus virginianus*) anesthetized with a phencyclidine-promazine (PHP) mixture. Because pre-immobilization excitement can cause release of plasma proteins from the liver and other reservoirs into circulation, high initial concentrations probably do not represent basal protein levels, as suggested by Wesson et al. (1979a). Subsequent samples represent a decrease in the direction of normal resting concentrations, caused by expansion of plasma volume with extracellular fluid resulting from the osmotic protein gradient (Seal et al., 1972; Wesson et al., 1979a). We recommend that blood samples for serum protein investigations be collected 60–90 min post-im-

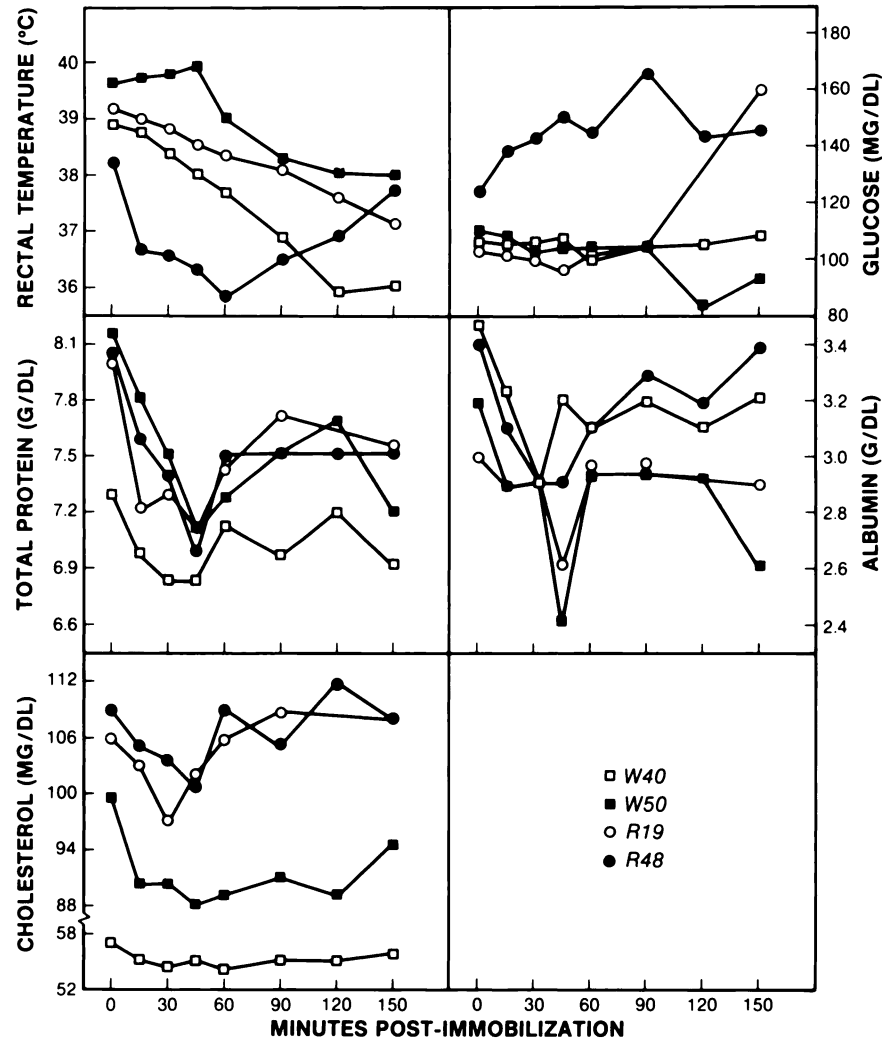


FIGURE 1. Rectal temperature and concentrations of serum protein, cholesterol, glucose and albumin in male (W40, W50) and female (R19, R48) peccaries subjected to serial bleeding under anesthesia with ketamine hydrochloride. Time from darting to immobilization ranged from 10 to 12 min.

mobilization in the collared peccary to permit osmotic equilibration.

Concentrations of serum urea nitrogen and creatinine did not vary significantly ( $P < 0.05$ ) with time post-immobilization (Table 1). These observations differ from the results of Gibson (1980), who demonstrated a significant rise in blood urea nitrogen at 90 min post-immobilization which would be attributed to pharmacological effects of xylazine administration.

Wesson et al. (1979a) stated that urea nitrogen is one of the most stable blood parameters with regard to short-term effects of blood collection. However, they warned that blood collected for urea nitrogen analysis should be obtained within 30 min of drug administration to guard against possible increases which may occur in association with the protein catabolic effects of elevated glucocorticoids. A similar recommendation can be made for the col-

TABLE 1. Serum chemistry values over time during anesthesia with ketamine hydrochloride in the collared peccary (values represent mean  $\pm$  SE).

Chemistry	Units	n	Minutes post-immobilization							
			0	15	30	45	60	90	120*	150
BUN <sup>b</sup>	mg/dl	4	14.5 $\pm$ 1.7	14.5 $\pm$ 1.6	15.0 $\pm$ 1.5	12.8 $\pm$ 1.4	14.7 $\pm$ 1.8	14.0 $\pm$ 1.2	14.7 $\pm$ 1.7	14.3 $\pm$ 0.9
Creatinine	mg/dl	4	1.2 $\pm$ 0.1	1.2 $\pm$ 0.1	1.1 $\pm$ 0.0	1.1 $\pm$ 0.2	1.2 $\pm$ 0.2	1.1 $\pm$ 0.2	1.1 $\pm$ 0.2	1.2 $\pm$ 0.1
Glucose	mg/dl	4	110 $\pm$ 4	114 $\pm$ 8	113 $\pm$ 10	114 $\pm$ 12	112 $\pm$ 11	120 $\pm$ 15	111 $\pm$ 17	127 $\pm$ 15
LDH <sup>c</sup>	IU/liter	4	1204 $\pm$ 53	1135 $\pm$ 51	1129 $\pm$ 55	1085 $\pm$ 58	1233 $\pm$ 118	1388 $\pm$ 261	1231 $\pm$ 16	1436 $\pm$ 236
GTT <sup>d</sup>	IU/liter	4	5.3 $\pm$ 1.0	5.3 $\pm$ 0.8	5.8 $\pm$ 0.9	6.5 $\pm$ 0.3	5.5 $\pm$ 0.9	5.3 $\pm$ 0.9	5.7 $\pm$ 0.3	5.8 $\pm$ 1.3
ALP <sup>e</sup>	IU/liter	4	26.0 $\pm$ 2.3	23.3 $\pm$ 1.3	22.0 $\pm$ 1.5	22.3 $\pm$ 2.0	22.8 $\pm$ 1.6	23.8 $\pm$ 1.3	22.0 $\pm$ 1.0	23.8 $\pm$ 1.5
Potassium	mmol/liter	4	4.2 $\pm$ 0.1	4.2 $\pm$ 0.1	4.1 $\pm$ 0.1	3.9 $\pm$ 0.2	4.0 $\pm$ 0.2	4.0 $\pm$ 0.2	3.9 $\pm$ 0.3	4.1 $\pm$ 0.2
Chloride	mmol/liter	4	106 $\pm$ 1	106 $\pm$ 1	105 $\pm$ 1	105 $\pm$ 1	106 $\pm$ 1	106 $\pm$ 1	106 $\pm$ 1	108 $\pm$ 1
Sodium	mmol/liter	4	146 $\pm$ 4	145 $\pm$ 1	144 $\pm$ 1	141 $\pm$ 1	142 $\pm$ 2	144 $\pm$ 1	145 $\pm$ 2	145 $\pm$ 2

\* n = 3.

<sup>b</sup> BUN = blood urea nitrogen.<sup>c</sup> LDH = lactic dehydrogenase.<sup>d</sup> GTT = gamma glutamyl transferase.<sup>e</sup> ALP = alkaline phosphatase.

lared peccary. Lochmiller and Grant (1984) reported significantly higher concentrations of serum urea nitrogen in trapped peccaries compared to shot peccaries.

#### Serum glucose and cholesterol

Serum glucose was not significantly ( $P > 0.05$ ) influenced by time post-immobilization. However, individual responses were variable (Fig. 1). Whereas female R48 showed a gradual increase over time, female R19 showed an abrupt increase in serum glucose. In comparison, males W40 and W50 had glucose concentrations which remained stable over time. The initial sample in the present study probably did not represent basal glucose concentrations as an adrenergic response to the stress of darting would have mediated an immediate rise in serum glucose. In addition, ketamine anesthesia has been associated with induction of a mild hyperglycemia, possibly mediated by impaired insulin activity or decreased hepatic metabolism of glucose (Hsu and Hembrough, 1982). Dyson (1969) reported a mean serum glucose level of 81 mg/dl for six peccaries immobilized with phencyclidine (PH) markedly lower than mean values reported in the present study and by Lochmiller and Grant (1984). Dyson (1969) did not give the length of time between immobilization and collection of samples. Elevated concentrations of serum glucose observed in females R19 and R48 between 90 and 150 min post-immobilization may have been associated with the long-term gluconeogenic effect of elevated glucocorticoids (Fig. 3). These results demonstrate the difficulty in determining basal glucose concentrations in the ketamine-immobilized peccary. Blood samples for glucose assessment should be collected as soon as possible following immobilization using standardized handling procedures.

Serum cholesterol concentrations varied

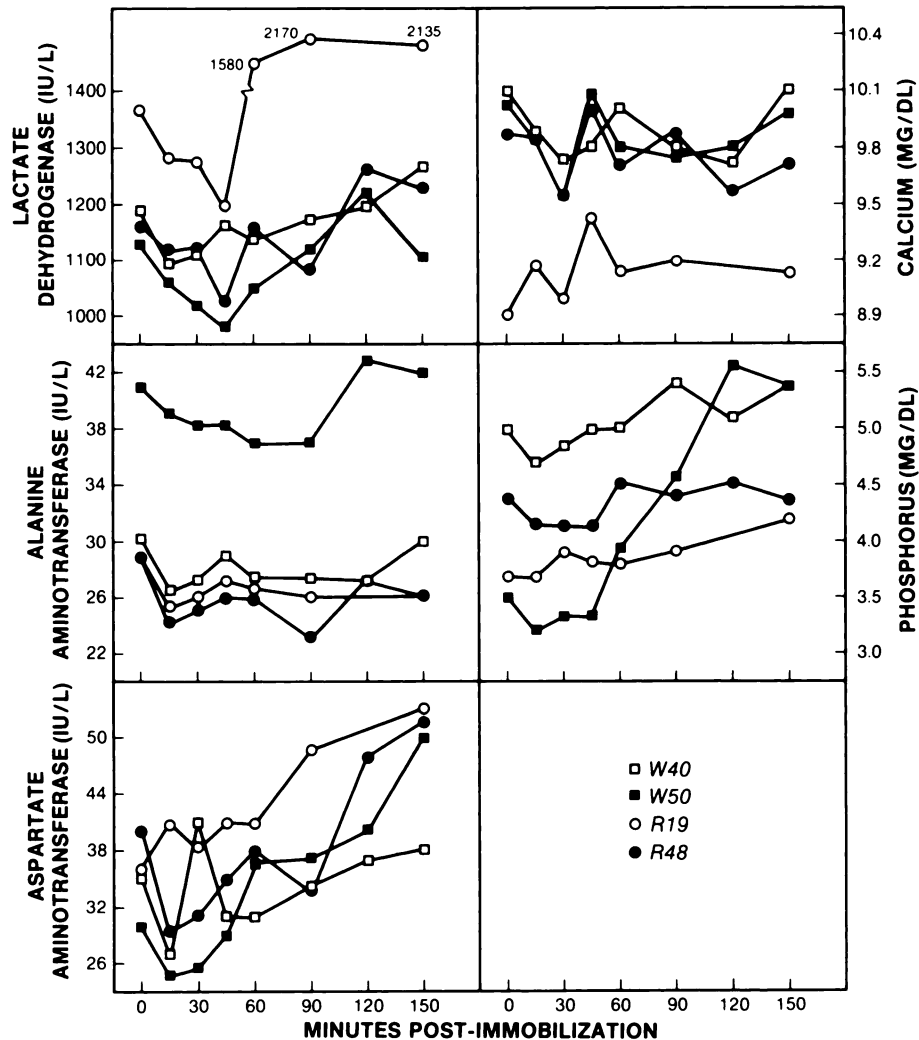


FIGURE 2. Concentrations of serum lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, calcium, and phosphorus in male (W40, W50) and female (R19, R48) peccaries subjected to serial bleeding under anesthesia with ketamine hydrochloride. Time from darting to immobilization ranged from 10 to 12 min.

significantly ( $P < 0.05$ ) with time post-immobilization, decreasing to minimal amounts by the 30-min sample, followed by a gradual increase to initial concentrations by 150 min post-immobilization (Fig. 1). Reports on the effect of immobilization on concentrations of serum cholesterol are conflicting, showing decreases (Seal et al., 1972) or increases (Franzmann, 1972). Lochmiller and Grant (1984) reported that

concentrations of serum cholesterol were higher in trapped, ketamine-anesthetized peccaries compared to shot animals. This suggests that blood samples to be assayed for serum cholesterol should be collected immediately after immobilization.

#### Serum enzymes

Concentrations of AST dropped initially, then rose significantly ( $P < 0.01$ ) to a

peak concentration at 150 min post-immobilization (Fig. 2). Similar observations have been made in other species (Franzmann and Thorne, 1970; Seal et al., 1972; Rehbindler and Edqvist, 1979) and associated with capture-induced myopathy (Gibson, 1980). In the present study, animals were not physically restrained before sampling and were darted with a minimum of muscle trauma. Trauma at the injection site and locally damaging effects of ketamine probably contributed to the increase in AST concentrations. Serum GGT activity was not elevated significantly ( $P > 0.05$ ) over time (Table 1).

Serum ALT concentration dropped significantly ( $P < 0.01$ ) within 15 min post-immobilization and returned to initial amounts by the end of the sampling period (Fig. 2). Mottelb (1980) demonstrated a significant rise in ALT in ketamine-anesthetized buffalo calves over 2 hr and suggested ketamine hepatotoxicity as the causative agent. Concentrations of ALP and LD were not affected significantly ( $P > 0.05$ ) by time post-immobilization (Table 1, Fig. 2), although they gradually decreased between 0 and 45 min following immobilization. The acute rise in concentrations of LD observed in female R19 (Fig. 2) may have been due to tissue trauma at the site of venipuncture. Hemodilution with extracellular fluid may be responsible for the initial decreases in serum enzyme concentrations. Based on these results, it is recommended that blood samples collected for assessment of serum enzyme activity in the collared peccary be collected soon after immobilization or 60 min post-immobilization to permit homeostatic recovery.

#### Serum minerals and electrolytes

Serum calcium concentrations declined to significantly ( $P < 0.01$ ) lower levels at 30 min post-immobilization before rebounding to values comparable to initial concentrations (Fig. 2). Changes in serum

calcium may be as a result of binding to serum albumin, which showed similar dynamics during the immobilization period (Fig. 1). Steyn (1974) found a similar decrease in calcium concentrations between 5 and 30 min post-immobilization followed by an increase between 30 and 60 min in male, but not female, Chacma baboons (*Papio ursinus*) anesthetized with PH. Concentrations of serum inorganic phosphorus were significantly ( $P < 0.05$ ) affected by time post-immobilization, with peak amounts observed at 120 min (Fig. 2). Male W50 showed a particularly dramatic rise in circulating phosphorus concentrations. Drevermo and Karstad (1974) found elevated phosphorus concentrations following xylazine anesthesia in impala (*Aepyceros melampus*). Serum electrolyte concentrations did not change significantly ( $P > 0.05$ ) with time post-immobilization despite an initial hemodilution (Table 1). These data suggest that serum minerals and electrolytes should be measured within 30 min post-immobilization when using ketamine.

#### Steroid hormones

Concentrations of serum glucocorticoid did not vary significantly ( $P > 0.05$ ) with time post-immobilization, although three of the four animals showed considerable increases in glucocorticoids following immobilization (Fig. 3). It is hypothesized that female R48 accommodated well to the darting stress, producing a brief but potent pulse of ACTH which caused a sharp elevation in serum glucocorticoids between 15 and 30 min post-immobilization. Female R19, which had higher initial values than R48, showed a rise in serum glucocorticoid concentrations between 30 and 45 min post-immobilization. Stress of injection apparently provoked a longer-term response in R19 than in R48. Male W50 showed a response similar to R19. Lack of a glucocorticoid response in male W40 suggested drug injection was

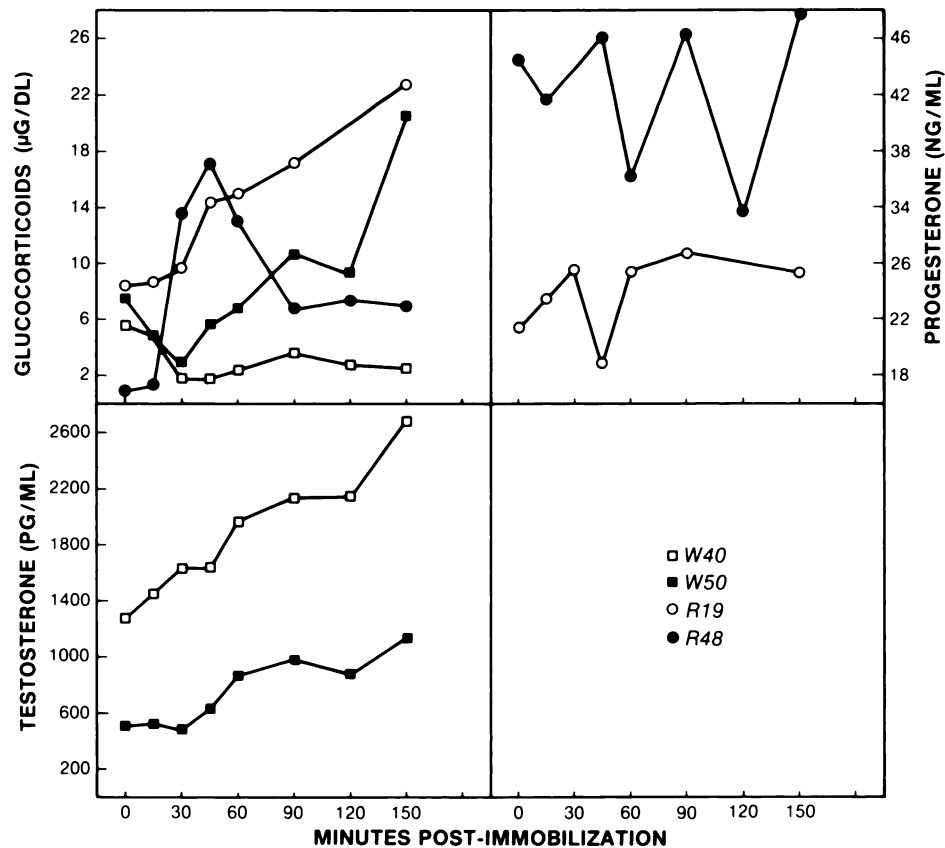


FIGURE 3. Concentrations of serum glucocorticoids, testosterone, and progesterone in male (W40, W50) and female (R19, R48) peccaries subjected to serial bleeding under anesthesia with ketamine hydrochloride. Time from darting to immobilization ranged from 10 to 12 min.

not perceived as a stressor and the hypothalamo-hypophyseal-adrenal axis was not activated. Fuller et al. (1984) reported that ketamine anesthesia does not alter the cortisol response to venipuncture in rhesus monkeys. Wesson et al. (1979b) observed a nonsignificant rise in mean plasma corticoids at 30, 90, and 150 min post-immobilization in white-tailed deer under PHP anesthesia.

Both males showed increases over time in serum testosterone (Fig. 3). Puri et al. (1981) found no change in serum testosterone following ketamine injection in rhesus monkeys, while stress associated with manual restraint did not cause elevated concentrations of testosterone in do-

mestic boars (Juniewicz and Johnson, 1984).

Serum progesterone concentrations of female peccaries did not change during the 150 min of post-immobilization monitoring (Fig. 3). Values were within reported ranges for pregnant peccaries (Sowls et al., 1976; Hellgren et al., 1985). Wesson et al. (1979b) found no change in plasma progestins in female white-tailed deer following PHP anesthesia. Plotka et al. (1983), however, found significant decreases in serum progesterone in pregnant deer following ketamine plus xylazine or promazine anesthesia and speculated that anesthesia depressed ACTH secretion and thus circulating progesterone of adrenal



origin. Rising concentrations of glucocorticoid in the peccaries in the present study suggested that endogenous ACTH release was not impaired. Future investigations should include assessment of the contribution of adrenal progesterone to circulating concentrations in the nonpregnant peccary. We suggest that blood samples collected for analysis of steroid hormones, particularly for serum glucocorticoid concentrations, should be collected within 15 min of immobilization in the collared peccary.

### Conclusions

Results of this study confirm and extend previous reports on effects of anesthesia and handling on circulating concentrations of serum constituents and steroid hormones. Acute side-effects of deep ketamine anesthesia include body temperature depression and slight hyperglycemia. Drug effects on serum enzymes remain poorly defined. Major effects of darting and handling were due to perception of stressful stimuli. Samples collected for determination of serum concentrations of glucose, cholesterol, urea nitrogen, creatinine, serum minerals, serum electrolytes, and steroid hormones should be obtained within 15 min to minimize stress-induced increases mediated by adrenergic and glucocorticoid action. Samples assayed for serum concentrations of total protein, albumin, AST, ALT, LD, and ALP should be collected 60 min following immobilization to permit osmotic equilibration, as these parameters show a rapid decline in circulating concentrations before stabilizing between 45 and 60 min post-immobilization. Handling procedures should be standardized to compare data within and between studies. Multiple samples should be taken over time if possible. Animals used in this study were captive and somewhat habituated to the experimentors' presence. Wild-trapped peccaries may react quite differently, particularly if they

have been in a trap for hours before sampling. Use of remote immobilization (Mech et al., 1984) or automatic blood samplers (Bubenik, 1983) would aid in obtaining normal baseline physiological data on free-ranging individuals.

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