The effect of capture stress and haloperidol therapy on the physiology and blood chemistry of springbok, *Antidorcas marsupialis*

by

M. D. Gericke

Department of Zoology, University of the Western Cape, Bellville, 7530

J. M. Hofmeyr

Nature Conservation and Tourism Division, South West African Administration

and

G. N. Louw

Zoology Department, University of Cape Town, Rondebosch, 7700

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ABSTRACT

In view of the alarmingly high losses which frequently occur as a result of capture myopathy during translocation of game animals, it was decided to investigate the main physiological and biochemical changes which occur during capture stress. In a series of experiments with eight springbok which were captured in drop nets, it was shown that a marked increase in rectal temperature (>42°C), cardiac rate ($\bar{x}$ = 159/min) and respiration rate ($\bar{x}$ = 120/min) occured. These changes were interpreted as being normal physiological adjustments to forced exercise, although the hyperventilation probably also partially compensated for a metabolic acidosis which is one of the features of capture stress. In addition it was found that under conditions of capture stress an extremely dramatic increase occured in plasma levels of glucose($\bar{x}$ = 221 mg/100ml), creatine kinase ($\bar{x}$ = 6582 mU/ml), lactate dehydrogenase ($\bar{x}$ = 38 349 mU/ml) and lactate ($\bar{x}$ = 190 mg/100 ml) with a concurrent reduction in blood pH (min. 6,748). These increases were far higher than those which occur during forced exercise in domestic animals such as the sheep and it became clear that a very intense alarm reaction occurs in captured springbok. In addition to the above changes, plasma levels of potassium rose significantly (max. 6,4 mEq/1) and fairly convincing evidence was obtained by means of gel electrophoresis that myoglobin was released into the plasma as a result of capture stress.

It was concluded that capture myopathy was caused in the present investigation by mechanical injury (trauma) and as a result of acute stress and hyperthermia brought about by forced exercise, which is moreover potentiated by an intense alarm reaction and a concomitant catecholamine release. Under the latter conditions haemoconcentration occurs, plasma glucose levels rise dramatically, and, in view of the increased work load on the muscles, the oxidative capacity of the mitochondria is exceeded. This results in hypoxia, increased anaerobic metabolism, a metabolic acidosis, an increase in lactate levels, and, of great importance, increased permeability of the cell membrane. The latter effect is the most probable cause for an efflux of various intracellular enzymes such as creatine kinase and lactate dehydrogenase as well as the electrolyte potassium. Potassium levels may increase to such an extent that normal myocardial function could be disrupted and this may be the ultimate cause of death in some cases. Of equal if not greater importance in this respect, however, is the loss of potassium from the intracellular fluid and particularly the muscle cells. This results in a disruption of normal cell function and consequent weakening of the muscles.

Preventative measures must therefore be aimed at reducing mechanical injury, the alarm reaction, hyperthermia and, as far as possible, avoiding over-exertion. In the present investigation considerable success was achieved by using improved capture techniques and

the tranquilliser haloperidol (G. D. Searle & Co.). In addition, supportive therapy should be directed towards alleviating the metabolic acidosis, promoting aerobic metabolism and thus restoring the integrity of the cell membrane.

1 INTRODUCTION

One of the more challenging problems in wildlife conservation today is that of overstraining disease or capture myopathy. Affected game animals may on occasion die suddenly without showing marked symptoms prior to death. Others sometimes excrete urine of a dark brown colour and often manifest clinical signs of muscular degeneration and pain. Post mortem examinations reveal the characteristic dull, whitish, necrotic muscular lesions which are frequently surrounded by extensive haemorrhages. These lesions occur in the skeletal as well as in the cardiac musculature. General congestion and severe pulmonary oedema, due to congestive heart failure, are observed in acute cases, as well as degenerative changes in the liver and kidneys (Young and Bronkhorst, 1971; Young, 1972).

It is well known that severe exercise in man causes certain changes in the blood chemistry. The acid-base balance is disturbed, anaerobic metabolites accumulate and the level of certain intracellular enzymes and electrolytes rise in the plasma. (Block *et al.*, 1969; Dancaster *et al.*, 1971; Fowler *et al.*, 1962).

Harthoorn and van der Walt (1974) have speculated that the extensive lesions of the muscles encountered during capture myopathy, are in fact a combination of the direct effect of lactic acid on the muscle fibres, combined with forced exercise of the muscle which tends to go into spasm as a result of the changes in the hydrogen ion concentration.

Hofmeyr, Louw and du Preez (1973) found that the blood chemistry of a chased group of zebras showed similarities to that of a zebra that died of shock. In both cases the plasma levels of glucose, lactate and creatine kinase were elevated. They were therefore tempted to conclude that incipient signs of the shock syndrome (capture myopathy) were already evident in the chased group of animals. Moreover, the general changes in the blood chemistry of the exercise-stressed zebras indicated that they displayed an alarm reaction and that anaerobic metabolism was employed. The enzyme levels in the plasma were also elevated. Since both the catecholamines and hypoxia are known to cause an increase in plasma enzymes, it seems reasonable to assume that these factors also contribute to the increased cellular membrane permeability during exercise-stress.

Very recently Harthoorn and Young (1976) have investigated several parameters, which may be involved in the stress syndrome associated with the capture of wild game. They are also of the opinion that the development of a marked metabolic acidosis, an increase in pulmonary arterial pressure and elevation of certain serum enzymes are involved in the syndrome generally known as capture myopathy.

It therefore seems that a thorough study of the effect of stress on blood chemistry may bring us closer to the solution of the overstraining disease problem. The purpose of the present investigation was therefore to take the studies of Hofmeyr *et al.* (1973) a step further by examining the effect of capture stress on the blood chemistry of springbok in more detail.

2 PROCEDURE

2.1 Experiment 1

During a springbok capturing expedition in July, 1973 on the farm Lochkolk in South West Africa, eight springbok were randomly selected for this experiment. The animals were caught in drop nets, after being herded by vehicles and a fixed-wing aircraft for approximately 15 – 20 minutes (Hofmeyr, Luchtenstein and Mostert, 1977). Immediately after they had been netted a heparinised blood sample was drawn from the external jugular vein of each animal, centrifuged within ten minutes and the plasma immediately frozen on solid CO_2 in the field for later analysis. Prior to centrifugation a sub-sample was taken for micro-haematocrit determination. In addition, a 2 ml heparinised venous blood sample was taken anaerobically for pH determination. This was frozen immediately and pH determinations were carried out within a week. After the blood samples had been taken the rectal temperature, cardiac rate and respiration rate were recorded and an injection (0,20 to 0,30 mg / kg) of haloperidol (Searle) was administered (Hofmeyr *et al.*, 1977).

The animals were then transported to a small enclosure (8 × 4 m hessian-lined boma) where they were kept for two weeks (Plate 1). After the first blood samples (stress samples) had been taken, the above blood sampling procedure was again repeated after 1 h, 6 h, 24 h, 48 h and 120 h. Samples for pH determinations were only collected up to 6 hours after stress. A further maintenance dose of haloperidol was administered 16 hours after the first dose, with the result that the animals were tranquillised and calm when the 1 h, 6 h and 24 h samples were taken. Once the tranquilliser effect wore off, the springbok had to be forcibly captured in the enclosure and restrained by hand. Hofmeyr *et al.* (1977) have shown that the tranquillising effect of haloperidol disappears within 10 – 12 hours. Nevertheless, it was not considered advisable to keep the springbok under the influence of haloperidol during the entire sampling period, as the drug may have influenced their feeding behaviour. Analysis of the plasma included determinations of plasma glucose, lactate, creatine kinase

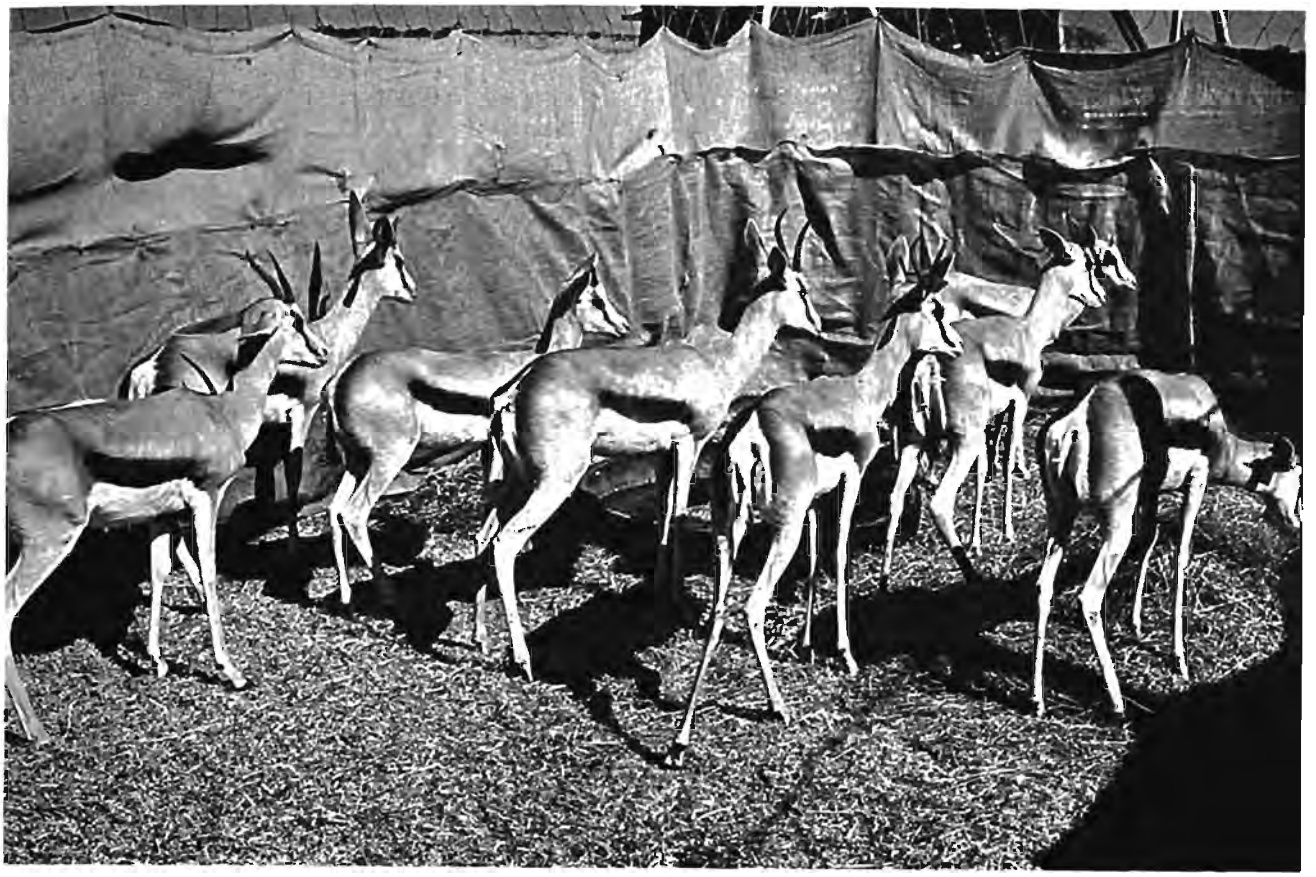

PLATE 1: The experimental animals in hessian-lined holding pen.

(C.P.K.), lactate dehydrogenase (L.D.H.), sodium, potassium, myoglobin and haemoglobin.

Boehringer test kits and an Eppendorf photometer model 1101 M were used for the determination of plasma glucose, lactate, C.P.K. and L.D.H. The test kit instructions were followed exactly. In the case of C.P.K. and L.D.H. the plasma had to be diluted ten times with physiological saline, because the optical density differences exceeded the maximum allowed. In the case of C.P.K., diluted plasma may have a 40 % higher activity than undiluted plasma. To correct for this higher activity, 40 % was subtracted from every C.P.K. value thus obtained.

To test if myoglobin and haemoglobin were present in the plasma, electrophoresis of pure myoglobin, pure haemoglobin and all the plasma samples were carried out by the cellulose acetate method on a Beckman microzone electrophoretic apparatus. This method was not sufficiently sensitive and therefore acrylamide gel electrophoresis was also carried out on a few plasma samples, with pure myoglobin and pure haemoglobin as standards. Sodium and potassium concentrations were determined, using standard flame photometric techniques (Instrumentation Laboratory, IL 343). Determinations of pH were carried out on an Astrup Radiometer and pH meter PHM 71 / BMS 2, at a temperature of 37° C. In order to establish if storage of the blood samples may have affected the pH, additional samples of sheep blood were collected anaerobically under identical conditions and the pH immediately determined. They were then frozen for two weeks and the pH re-determined. The change in pH after freezing was found to be negligible. Statistical analysis of the data consisted of testing for significance between sampling periods by using Student's t test.

2.2 Experiment 2

In an attempt to collect control blood samples, eight springbok were shot separately at a water hole from a concealed position on the farm Rooipoort in the Kimberley district. All of them received headshots and they died immediately, apparently without the slightest alarm. Within two minutes the thoracic cavity was opened and blood samples were drawn from the left ventricle for pH determinations, haematocrit and plasma analyses. These samples were treated in exactly the same way as previously described. We presumed that under these circumstances suitable samples would be obtained for control values. This, however, was later proved not to be the case.

3 RESULTS AND DISCUSSION

3.1 Experiment 1

3.1.1. Cardiac rate, respiration rate and rectal temperature

Capturing had a very pronounced effect on the cardiac rate. Table 1 shows the highly significant decrease in cardiac rate after one hour, with a further significant decrease six hours later. One can assume that this approximates the normal cardiac rate, because twenty-four hours after capture the same value was obtained. Later values showed another increase in the cardiac rate, which can be ascribed to the fact that the animals were no longer tranquillised and again displayed an alarm reaction.

Various factors contribute to the rise in cardiac rate during exercise. They include sympathetic nerve stimulation, a rise in the carbon dioxide content and temperature of the blood, perhaps acid metabolites, and, very important – the catecholamines secreted by the adrenal medulla (Karpovich, 1959; Morehouse *et al.*, 1967). With regard to the latter it should be noted that the springbok were not accustomed to humans or to herding and displayed an intense alarm reaction.

TABLE 1: Effect of capture and subsequent haloperidol therapy on cardiac rate.

CARDIAC RATE (rate / min)				
GROUP	MEAN	RANGE	S.D.	N
STRESSED	160	120–200	± 32	7
1 Hour	96	76–116	± 16	8
6 Hours	79	72– 92	± 7	8
24 Hours	80	60– 96	± 12	8
48 Hours*	109	80–132	± 22	8
120 Hours*	112	86–138	± 20	8

Stressed vs. 1 Hour	–	H.S. (P <0,001)
1 Hour vs. 6 Hours	–	S. (P <0,025)
6 Hours vs. 24 Hours	–	N.S. (P >0,05)
24 Hours vs. 48 Hours	–	H.S. (P <0,005)
48 Hours vs. 120 Hours	–	N.S. (P >0,05)

* Not under influence of haloperidol

The marked increase in respiration rate after stress had disappeared after one hour and no significant changes occurred later (Table 2). This elevation in respiration rate represents normal physiological adjustment to the demands of forced exercise. In this way oxygen supply to the muscles is increased and, concurrently, any metabolic acidosis which may develop is partially compensated for by hyperventilation.

Unfortunately the thermometer used for the experiments could only register values up to 42° C. The stressed springbok frequently exhibited a temperature in excess of 42° C after exercise and this has been indicated as 42+. No statistical analysis was therefore possible on the rectal temperature values.

TABLE 2: Effect of capture and subsequent haloperidol therapy on respiration rate.

RESPIRATION RATE (rate / min)				
GROUP	MEAN	RANGE	S.D.	N
STRESSED	120	62–158	± 36	7
1 Hour	26	14– 44	± 9	8
6 Hours	23	18– 32	± 5	8
24 Hours	26	12– 40	± 9	8
48 Hours*	33	24– 52	± 10	8
120 Hours*	50	26– 94	± 22	8

Stressed vs. 1 Hour	–	H.S. (P <0,001)
1 Hour vs. 6 Hours	–	N.S. (P >0,05)
6 Hours vs. 24 Hours	–	N.S. (P >0,05)
24 Hours vs. 48 Hours	–	N.S. (P >0,05)
48 Hours vs. 120 Hours	–	N.S. (P >0,05)

* Not under influence of haloperidol

Directly after stress, four of the eight animals had a temperature greater than 42° C and the minimum value was 40,8, while only one animal registered a temperature in excess of 42° C one hour later and the minimum was 39,3° C. The individual results indicate that the rectal temperature decreased appreciably after one hour. Subsequent readings never included a temperature greater than 42° C and the mean values are given in Table 3.

TABLE 3: Effect of capture and subsequent haloperidol therapy on rectal temperature.

RECTAL TEMPERATURE (°C)			
GROUP	MEAN	RANGE	N
STRESSED	–	40,8–42 +	8
1 Hour	–	39,3–42 +	8
6 Hours	38,8	38,1–39,4	8
24 Hours	38,9	38,2–39,7	8
48 Hours	39,9*	39,4–40.2	8
120 Hours	39,5*	38,8–40,0	8

* Not under influence of haloperidol

The increase in rectal temperature indicates that the animals did not fully succeed in dissipating the excess heat produced during exercise. However, they were all able to tolerate this high body temperature and hyperthermia was no longer evident after six hours. Neither Harthoorn and van der Walt (1974) nor Hofmeyr *et al.* (1973) found such high temperatures after capture in either the blesbok or zebra.

3.1.2. Haematocrit

Table 4 shows that there was no significant decrease in the high haematocrit values obtained immediately after capture until the 120 h observation.

TABLE 4: Effect of capture and haloperidol therapy on haematocrit values.

	HAEMATOCRIT			
GROUP	MEAN	RANGE	S.D.	N
STRESSED	52	47–56	± 3,4	8
1 Hour	49	43–54	± 4,7	8
6 Hours	47	42–52	± 4,0	8
24 Hours	47	40–53	± 4,3	8
48 Hours*	50	45–55	± 3,7	8
120 Hours*	43	35–48	± 4,1	8

Stressed vs. 1 Hour	–	N.S. (P >0,05)
1 Hour vs. 6 Hours	–	N.S. (P >0,05)
6 Hours vs. 24 Hours	–	N.S. (P >0,05)
24 Hours vs. 48 Hours	–	N.S. (P >0,05)
48 Hours vs. 120 Hours	–	H.S. (P <0,005)

* Not under influence of haloperidol

The following factors may have contributed to an apparently increased haematocrit value found after stress, namely an intense sympathetic reaction involving catecholamine release and contraction of the splenic capsule (Guyton, 1968) or a reduction in plasma volume. Haemoconcentration and dilution in exercise has been extensively studied (Costill *et al.*, 1974; Dill *et al.*, 1974; Hofmeyr *et al.*, 1973).

Hofmeyr *et al.* (1973) found in their experiments on zebra that the haematocrit values fell within one hour. It is difficult to explain the difference between their findings and those of the present investigation. In fact, until reliable normal values have been established, further discussion in this respect remains speculative. We are, however, inclined to accept the high values obtained in this investigation, particularly when viewed with the dramatic hyperglycemia, as being an index of an alarm reaction and catecholamine release. This view is strengthened by the observation that when one springbok was darted with etorphine hydrochloride / M99 (Reckitt) and azaperone (Janssen Pharmaceutica) without inducing any overt alarm reaction, the haematocrit values remained at 40 – 42 % throughout a three-hour immobilisation period. The low values recorded at 120 hours were, nevertheless, obtained after recapture without tranquilliser treatments and should theoretically have paralleled the high glucose values obtained. The only rational explanation that we can offer for this phenomenon is possible intravascular haemolysis.

3.1.3. Plasma levels of glucose and lactate

In Figure 1 and Table 5 it can be seen that capture resulted in a very high glucose level in the plasma, which prevailed for a considerable time. The first significant decrease occurred at 24 h. However, the 48 and 120 h values were elevated again.

The extremely high glucose values observed after stress in the present investigation indicate that the catecholamine reaction appears to have been very intense although without actual measurements of circulating levels of catecholamines this conclusion cannot be made with absolute certainty.

The reason why the glucose remained at a high level for a considerable period is, however, not clear, although the effect of cortico-steroids in gluconeogenesis may have been significant in this respect. The rise in the glucose level at 48 and 120 h, when the animals were no longer tranquillised, can probably be ascribed to additional alarm reactions.

TABLE 5: Effect of capture and haloperidol therapy on the plasma levels of glucose.

	GLUCOSE CONCENTRATION (mg / 100 ml)			
GROUP	MEAN	RANGE	S.D.	N
STRESSED	198,3	116,9–237,0	± 37,7	8
1 Hour	195,0	148,6–232,9	± 31,5	8
6 Hours	221,5	102,5–291,3	± 55,7	8
24 Hours	118,0	86,3–153,8	± 20,5	8
48 Hours*	159,0	116,8–240,8	±39,5	8
120 Hours*	167,2	147,8–237,2	± 29,2	8

Stressed vs. 1 Hour	–	N.S. (P >0,05)
1 Hour vs. 6 Hours	–	N.S. (P >0,05)
6 Hours vs. 24 Hours	–	H.S. (P <0,001)
24 Hours vs. 48 Hours	–	S. (P <0,025)
48 Hours vs. 120 Hours	–	N.S. (P >0,05)

* Not under influence of haloperidol

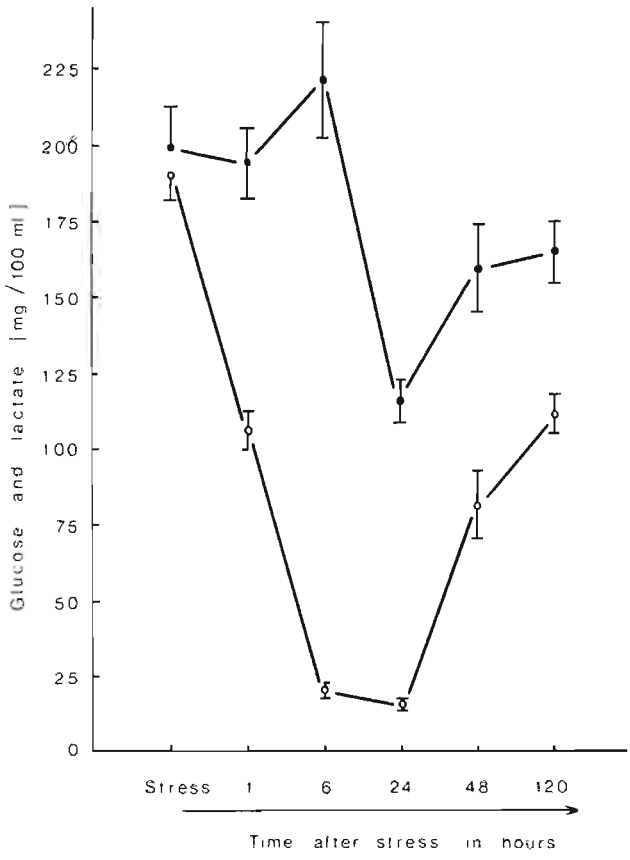

FIGURE 1: Effect of capture and haloperidol therapy on plasma levels of glucose and lactate (N = 8).
● Glucose concentrations ± S.E.
○ Lactate concentrations ± S.E.

Table 6 and Fig. 1 show that the very high lactate levels observed after stress returned to normal within 6 h. The plasma lactate levels exhibited by these animals after stress were much higher than the value of 68,9 ± 6,4 recorded by Hofmeyr *et al.* (1973) for chased zebra. It can be assumed, therefore, that the springbok experienced greater stress and had to employ anaerobic metabolism to a much greater extent.

The high lactate level in the 48 and 120 h samples, when the animals were no longer tranquillised, is also of interest. They were kept in a small boma, and most of them struggled for one or two minutes before the 48 and 120 h blood samples could be taken. Keppler, Keul and Doll (1969) found that during a 60-second work period, muscle glycogenolysis seems to be maximally activated and surpasses the mitochondrial capacity for pyruvate oxidation. In this experiment the animals also showed an intense fear reaction when the untranquillised samples were collected at 48 and 120 h, thus explaining the high lactate levels found after a struggle of only one or two minutes. It would also seem as if haloperidol therapy was instrumental in reducing lactate levels very effectively.

TABLE 6: Effect of capture and haloperidol therapy on the plasma levels of lactate.

LACTATE CONCENTRATION (mg / 100 ml)				
GROUP	MEAN	RANGE	S.D.	N
STRESSED	190,1	145,9–211,3	± 22,2	8
1 Hour	107,8	75,9–129,1	± 19,5	8
6 Hours	20,9	14,0–24,6	± 3,4	8
24 Hours	17,0	9,6– 22,4	± 4,6	8
48 Hours*	82,3	36,8–127,8	± 33,7	8
120 Hours*	112,6	89,1–139,5	± 18,9	8
Stressed vs. 1 Hour	–	H.S. (P <0,001)		
1 Hour vs. 6 Hours	–	H.S. (P <0,001)		
6 Hours vs. 24 Hours	–	N.S. (P >0,05)		
24 Hours vs. 48 Hours	–	H.S. (P <0,001)		
48 Hours vs. 120 Hours	–	S. (P <0,05)		

* Not under influence of haloperidol

3.1.4. Blood pH values

Unfortunately only the pH values could be determined in this experiment and not the complete acid-base balance. However, on the basis of the respiration rate and lactate values observed, some conclusions can be drawn. For example, immediately after exercise the animals exhibited an extremely low blood pH (Table 7). Karpovich (1959) stated that the lowest pH of venous blood ever recorded in living man, at that time, was 6,78 after exercise. The animals were definitely in a condition of metabolic acidosis and the lactate concentration was very high. They also responded with hyperventilation, but it is not possible to say to what extent this affected the blood pH. It would seem, however, as if the animals showed a partially compensated metabolic acidosis immediately after exercise.

TABLE 7: Effect of capture and haloperidol therapy on blood pH.

pH				
GROUP	MEAN	RANGE	S.D.	N
STRESSED	7,033	6,748–7,262	± 0,159	7
1 Hour	7,248	7,135–7,305	± 0,058	8
6 Hours	7,262	7,200–7,331	± 0,055	8
Stressed vs. 1 Hour	–	H.S. (P <0,005)		
1 Hour vs. 6 Hours	–	N.S. (P >0,05)		

Two animals exhibited a blood pH below 7,0 immediately after exercise and it is of considerable significance that the lactate levels and respiration rate in these animals were among the highest recorded (Table 8).

TABLE 8: Respiration rate and plasma lactate concentration of two springbok, with a blood pH below 7,0.

ANIMAL NO.	pH	LACTATE mg / 100 ml	Resp. Rate
No. 29	6,748	200	106
No. 32	6,950	207,6	158

One hour after capture there was a highly significant increase in the mean blood pH values of the whole group and the 6 h value was still higher, although it did not differ significantly from the one-hour value. The mean value for blood pH at 6 h is still considerably lower than the normal pH of 7,35 for sheep and human venous blood. At this stage the respiration rate and lactate values had returned to normal. These results are in general agreement with those obtained by Harthoorn and Young (1976) for zebra and wildebeest and are comparable with results obtained by Harthoorn and van der Walt (1974) in blesbok.

3.1.5. Plasma levels of sodium, potassium, lactate dehydrogenase (L.D.H.) and creatine kinase (C.P.K.)

The sodium level (Table 9) showed a highly significant decrease one hour after the initial stress. Although the sodium values recorded at 6, 24 and 48 h are somewhat higher than the normal values for humans (136 – 148 mEq / l, Baron 1973), there was no statistically significant change until the elevation recorded at 120 h.

Capture appears to have caused a marked increase in the potassium levels immediately after stress (Table 10). One hour later the potassium level had already returned to normal, and no further significant changes occurred. However, some individuals showed a very low potassium concentration at various times after stress.

Metivier (1969), McKechnie, Leary and Joubert (1967) and Dancaster and Whereat (1971) also found a rise in potassium concentration after exercise, while McKechnie, Reid and Joubert (1970) found no rise in potassium concentration in marathon runners immediately after the event. Metivier (1969) found an increase in sodium levels after exercise, while Dancaster *et al.* (1971) found no difference in plasma sodium levels after a marathon run.

PLATE 2: Post mortem appearance of a springbok which exhibited severe hyperkalaemia (8,5 mEq/l) and grossly elevated plasma levels of C.P.K. (4 911 mU/ml) and L.D.H. (4 868 mU/ml) one hour after capture. Note extensive superficial haemorrhages and contusions.

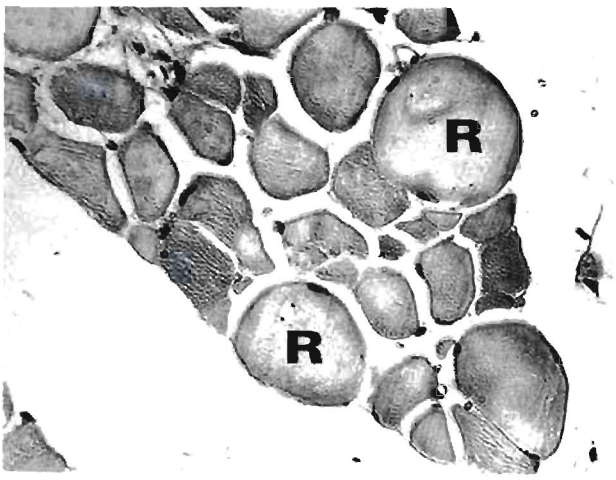

PLATE 3: Section of skeletal muscle showing rounded fibres which are indicative of hypoxia (R – rounded fibres).

Metivier (1969) is of the opinion that the increase in electrolytes in the serum during physical exercise, is a result of increased cellular permeability, brought about by catecholamine release. The efflux of potassium from the cells is further enhanced by the metabolic acidosis when hydrogen ions displace potassium ions from the cells. For example, Lade and Brown (1963) have shown that skeletal muscle in dogs loses potassium under conditions of respiratory acidosis and, equally important, cardiac muscle gains potassium under the same conditions.

The importance of mechanical trauma in causing an efflux of potassium ions from the muscle cells during capture operations using nets should not be underestimated. This impression was confirmed when one springbok exhibited potassium plasma levels as high as 8,5 mEq / l one hour after capture. This animal eventually died six hours after capture and post mortem examination revealed extensive superficial muscular contusions and subcutaneous haemorrhages (Plate 2). Histological examination of the skeletal muscle of the same animal showed rounding of the fibres which is indicative of hypoxia (Plate 3) (Röhm, 1973).

It would seem, therefore, that under conditions of capture stress, the loss of potassium from the intracellular fluid is probably brought about by catecholamine release, and metabolic acidosis resulting in increased membrane permeability and replacement of potassium ions by hydrogen ions, as well as by trauma and, possibly, the effects of adreno corticoid hormones. Moreover, these effects are of great significance, as they not only constitute a danger of cardiac arrest under conditions of hyperkalaemia, but the loss of potassium ions from the muscle cells is probably of even greater significance (Lade and Brown, 1963; Baron, 1973).

TABLE 9: Effect of capture and haloperidol therapy on the plasma levels of sodium.

SODIUM CONCENTRATION (mEq / l)				
GROUP	MEAN	RANGE	S.D.	N
STRESSED	165	160–170	± 3,3	8
1 Hour	147	127–162	± 13,2	8
6 Hours	155	151–158	± 2,2	8
24 Hours	156	151–164	± 4,1	8
48 Hours*	153	149–156	± 3,0	8
120 Hours*	161	153–168	± 4,8	8
Stressed vs. 1 Hour	–	H.S. (P <0,005)		
1 Hour vs. 6 Hours	–	N.S. (P >0,05)		
6 Hours vs. 24 Hours	–	N.S. (P >0,05)		
24 Hours vs. 48 Hours	–	N.S. (P >0,05)		
48 Hours vs. 120 Hours	–	H.S. (P <0,001)		

* Not under influence of haloperidol

Tables 11 and 12 and Figs 2 and 3 show that the plasma concentrations of C.P.K. and L.D.H. also increased dramatically after the initial stress. C.P.K. shows an early peak at 6 h and another at the 120 h sampling period at which time the animals were not under sedation. L.D.H. did not reach a peak until 48 h after stress. This pattern of elevation conforms with the generally accepted theory on elevation of serum enzyme levels found after cardiac infarction (Baron, 1973).

TABLE 10: Effect of capture and haloperidol therapy on the plasma levels of potassium.

POTASSIUM CONCENTRATION (mEq / l)				
GROUP	MEAN	RANGE	S.D.	N
STRESSED	5,1	4,2–6,4	± 0,75	8
1 Hour	4,1	3,3–4,9	± 0,63	8
6 Hours	3,8	2,9–4,3	± 0,54	8
24 Hours	3,7	2,8–4,4	± 0,46	8
48 Hours*	4,2	3,1–5,5	± 0,87	8
120 Hours*	4,3	2,5–5,2	± 0,98	8

Stressed vs. 1 Hour	–	S. (P <0,025)
1 Hour vs. 6 Hours	–	N.S. (P >0,05)
6 Hours vs. 24 Hours	–	N.S. (P >0,05)
24 Hours vs. 48 Hours	–	N.S. (P >0,05)
48 Hours vs. 120 Hours	–	N.S. (P >0,05)

* Not under influence of haloperidol

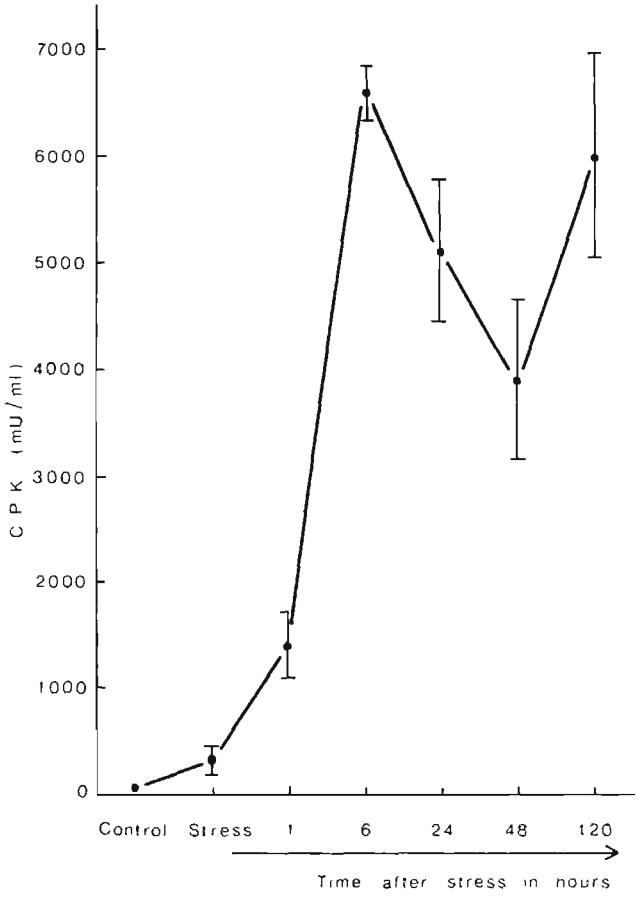

FIGURE 2: Effect of capture on the plasma levels of creatine kinase (C.P.K.) ± S.E. (N = 8).

Elevated C.P.K. levels have been recorded under similar circumstances by Block *et al.* (1969), McKechnie *et al.* (1970) and Hofmeyr *et al.* (1973). While increased L.D.H. levels have been observed by Novosadová (1969), Block *et al.* (1969), Altland and Highman (1961), Garbus, Highman and Altland (1964)

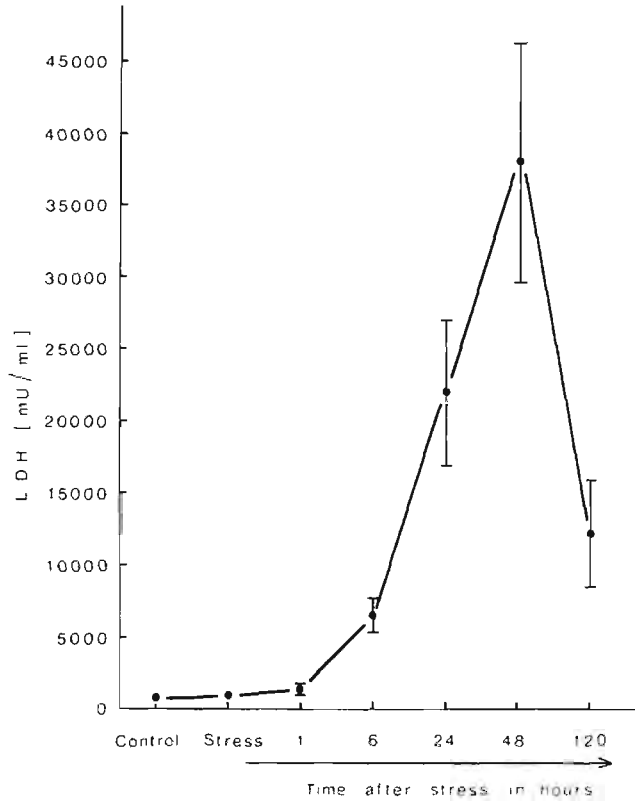

FIGURE 3: Effect of capture on the plasma levels of lactate dehydrogenase (L.D.H.) ± S.E. (N = 8).
* obtained from Experiment 2.

and McKechnie *et al.* (1967), the general opinion today is that increased cellular permeability results in increased plasma levels of intracellular enzymes. The reason why the cellular permeability increases can, however, be explained in various ways. For example, Highman, Mal'ng and Thompson (1959) found, with their studies on dogs, that intravenous infusion of either adrenalin or noradrenalin resulted in an elevation of serum G.P.T., serum G.O.T. and serum alkaline phosphatase. Adrenalin apparently has a more pronounced effect on the elevation of G.P.T. and alkaline phosphatase. Zierler found in his *in vitro* experiments on excised rat diaphragm (1956) and on the peroneus longus muscle of rats (1957) that the efflux of aldolase from the muscle is increased by anoxia and a high pottassium concentration and reduced by a reduction in temperature and by addition of glucose.

Highman and Altland (1960) have also demonstrated the effect of hypoxia on the increase of L.D.H. in blood serum in dogs, which were exposed to conditions of oxygen deprivation in a barochamber. Harthoorn and Young (1976) have also recorded elevated serum enzyme levels in wildebeest and zebra exposed to forced exercise in conjunction with increased pulmonary arterial pressure and a metabolic acidosis.

Fowler *et al.* (1962) uses indirect evidence, namely that the rise in enzymes is greater in untrained subjects than in trained subjects at progressively higher levels

of energy expenditure when anaerobic conditions prevail, to postulate that hypoxia might well be the principal factor causing increased membrane permeability. At a given work load, less well-conditioned subjects would probably suffer muscle hypoxia earlier than would trained or better-conditioned subjects whose blood flow through muscle is greater in either the resting or working state.

Thus we have evidence that both catecholamines and hypoxia *per se* have a direct effect on the leakage of the enzymes from the cells. It is also well known that hypoxia causes a catecholamine reaction in the body. Becker and Kreuzer (1969) found that hypoxia in general activates the sympatho-adrenal system in man, as evidenced by the increased catecholamine secretion. The ratio in which the medullary and nervous portions respond will depend on the nature of the other stress factors. Muscular exercise is primarily accompanied by an increased noradrenalin secretion, and emotional stress by increased adrenalin from the adrenal medulla.

Schmidt and Schmidt (1969) are of the opinion that it is likely that the enzymes remain within the cell compartments at their sites of action, as long as sufficient energy is supplied to maintain the structural order. It is well known that disturbances of the cellular metabolic efficiency, which reduce energy output, cause morphological modifications and enzyme release as well.

In this experiment the extremely high glucose levels and apparently high haematocrit values suggest that the animals experienced an intense alarm reaction with a concomitantly high adrenalin secretion rate. The high lactate level and low pH show that muscular activity was very intense and that a marked hypoxia, acidosis and anaerobic metabolism were experienced. These factors together with mechanical trauma would then explain the increased cellular permeability and tremendously high enzyme levels in the plasma.

TABLE 11: Effect of capture and haloperidol therapy on the plasma levels of creatine kinase (C.P.K..).

C.P.K. CONCENTRATION (mU / ml)				
GROUP	MEAN	RANGE	S.D.	N
CONTROL	28	24– 47	± 9	8
STRESSED	297	71–1206	± 377	8
1 Hour	1427	733–3262	± 822	8
6 Hours	6583	5815–7611	± 621	8
24 Hours	5117	2340–7398	± 1848	8
48 Hours*	3900	1796–7044	± 1972	8
120 Hours*	6035	3451–7847	± 2615	8

Control vs. Stressed	–	N.S. (P >0,05)
Stressed vs. 1 Hour	–	H.S. (P <0,005)
1 Hour vs. 6 Hours	–	H.S. (P <0,001)
6 Hours vs. 24 Hours	–	N.S. (P >0,05)
24 Hours vs. 48 Hours	–	N.S. (P >0,05)
48 Hours vs. 120 Hours	–	N.S. (P >0,05)

* Not under influence of haloperidol

TABLE 12: Effect of capture and haloperidol therapy on the plasma levels of lactate dehydrogenase (L.D.H.).

L.D.H. CONCENTRATION (mU / ml)				
GROUP	MEAN	RANGE	S.D.	N
CONTROL	766	637– 955	± 116	8
STRESSED	866	684– 1289	± 223	8
1 Hour	1222	803– 1909	± 364	8
6 Hours	6473	3007–13172	± 3576	8
24 Hours	21635	9068–33408	±14400	8
48 Hours*	38349	11995–80655	±23686	8
120 Hours*	12393	1623–33169	±10109	8

Control vs. Stressed	–	N.S. (P >0,05)
Control vs. 1 Hour	–	H.S. (P <0,005)
Stressed vs. 1 Hour	–	S. (P <0,05)
1 Hour vs. 6 Hours	–	H.S. (P <0,005)
6 Hours vs. 24 Hours	–	S. (P <0,025)
24 Hours vs. 48 Hours	–	N.S. (P >0,05)
48 Hours vs. 120 Hours	–	S. (P <0,025)

* Not under influence of haloperidol

3.1.6. Plasma colour – haemoglobin and myoglobin in the plasma

The plasma of the majority of springbok showed a distinct red-brown discolouration after stress which persisted until the 120 h sampling period (Plate 4).

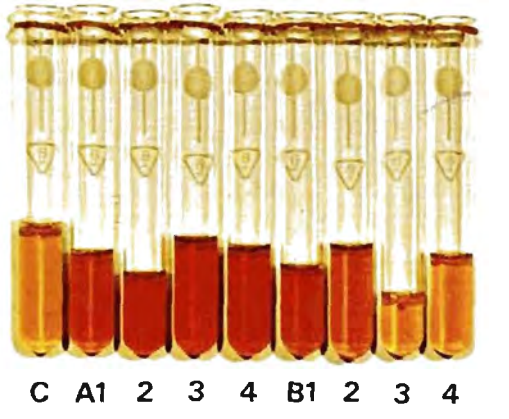

PLATE 4: Plasma samples of two springbok, immediately after capture, 1 h, 6 h, 24 h later, compared with a normal control sample. *C* – Control; *1* – Immediately after capture; 2 – One hour later; 3 – Six hours later; 4 – Twenty-four hours later; A and B – indicate two different individuals.

Harthoorn *et al.* (1974) found during their experiment on chased blesbok that only one animal, which had run for more than 10 km, displayed this red discolouration of the plasma. He presumed that this colour was due to liberated "myohaemoglobin". Young (1972) also described myoglobinuria as a clinical sign during overstraining disease in tsessebe.

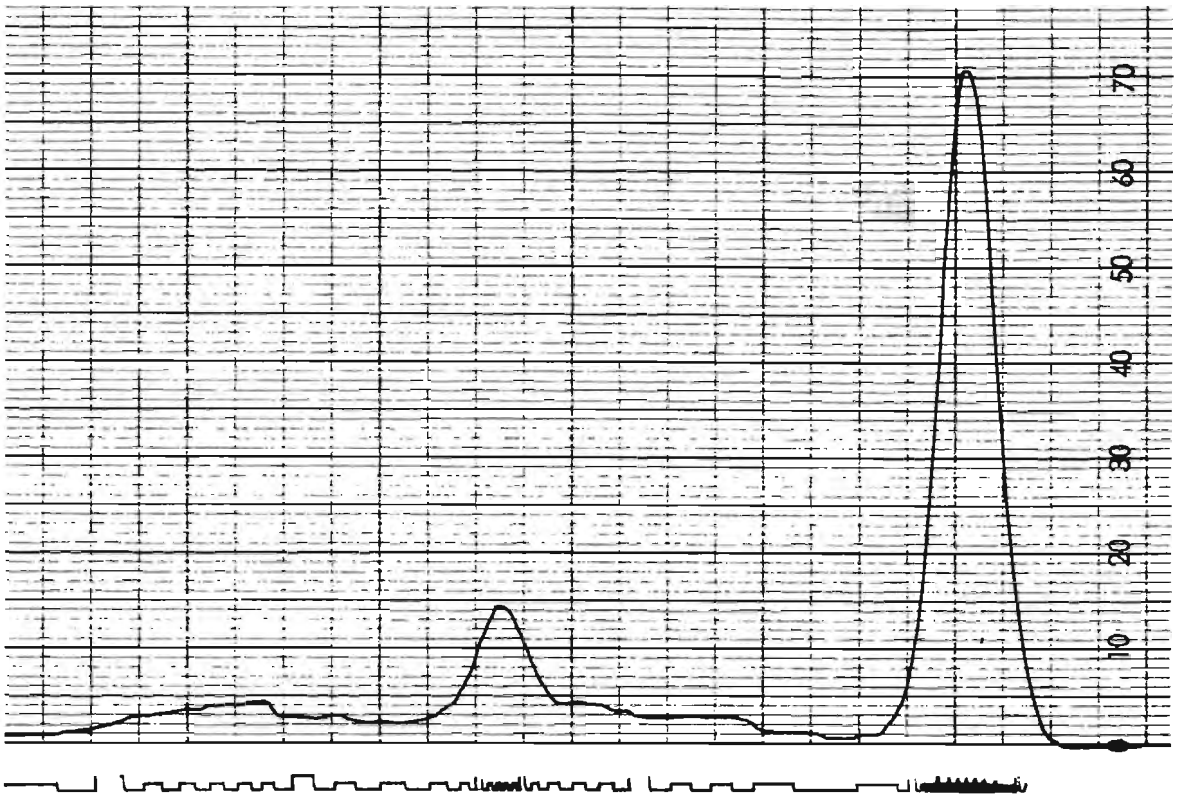

FIGURE 4: Normal electrophoretic pattern of plasma proteins in springbok no. 30, one hour after capture.

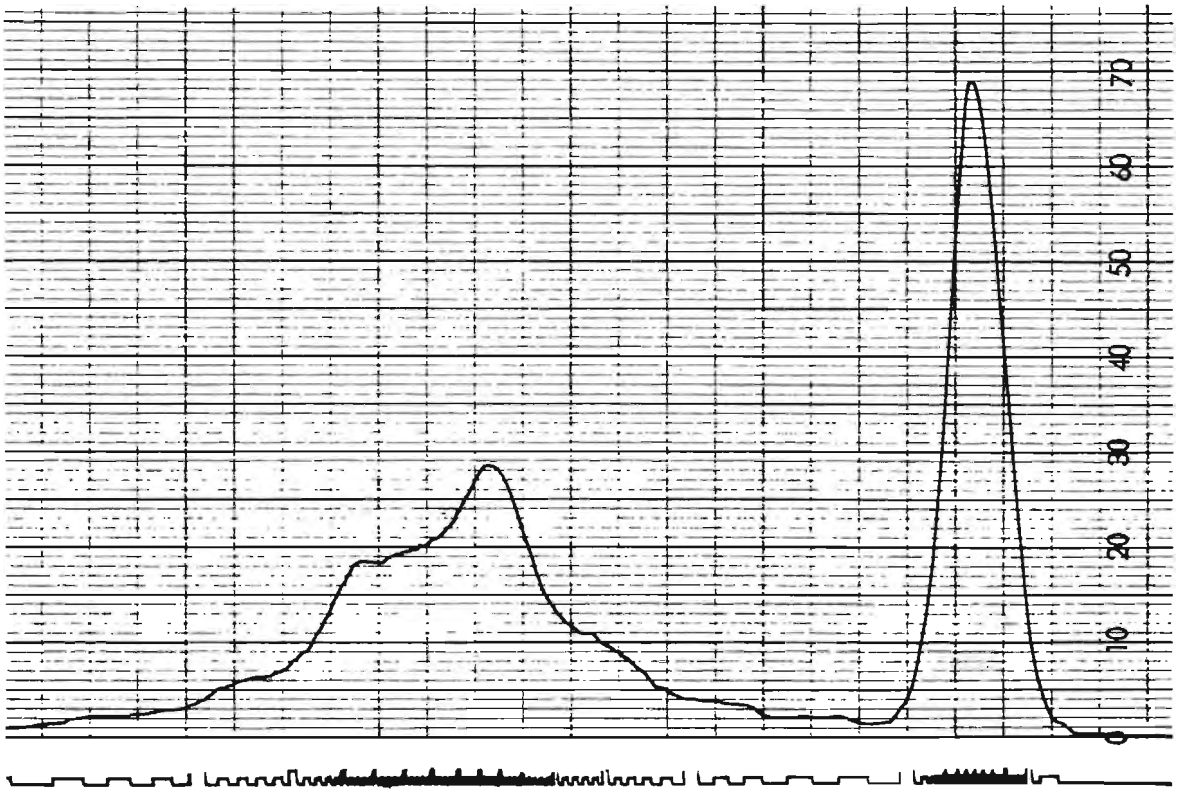

FIGURE 5: Electrophoretic pattern of pure haemoglobin, mixed with the one hour plasma sample of springbok no. 30.

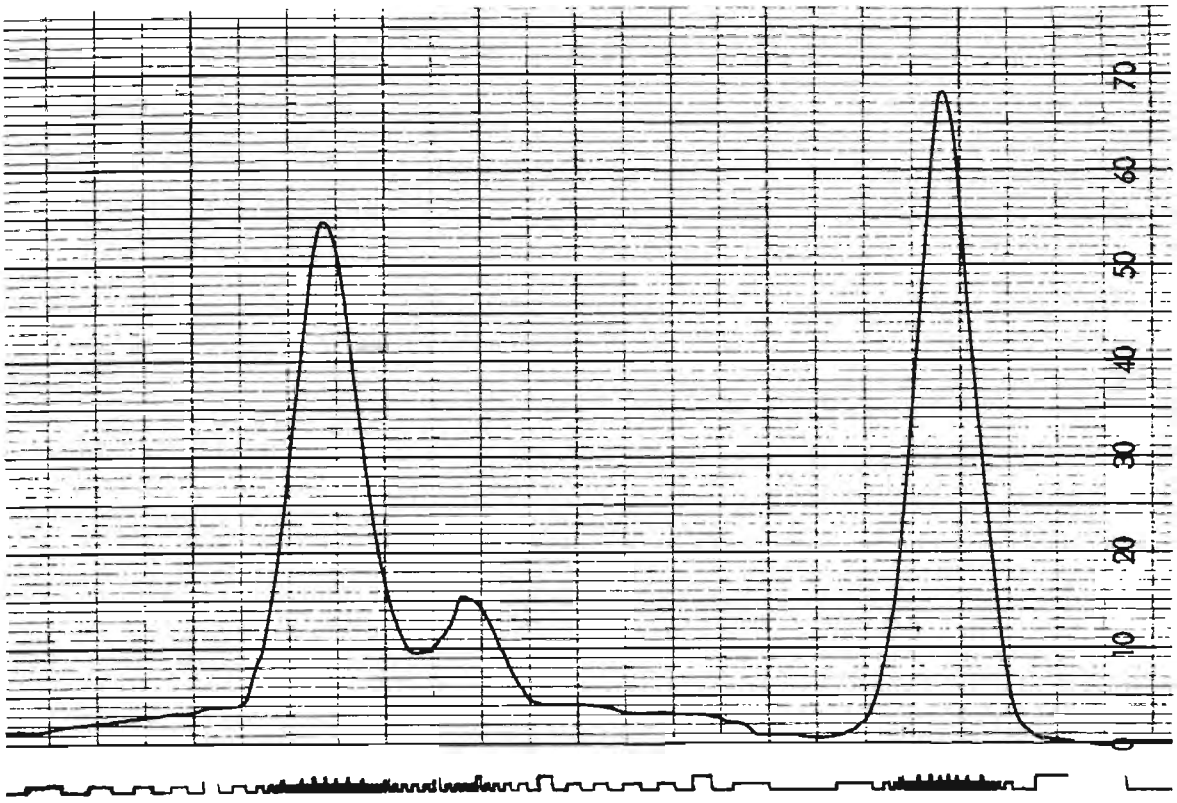

FIGURE 6: Electrophoretic pattern of pure myoglobin, mixed with the one hour plasma sample of springbok no. 30.

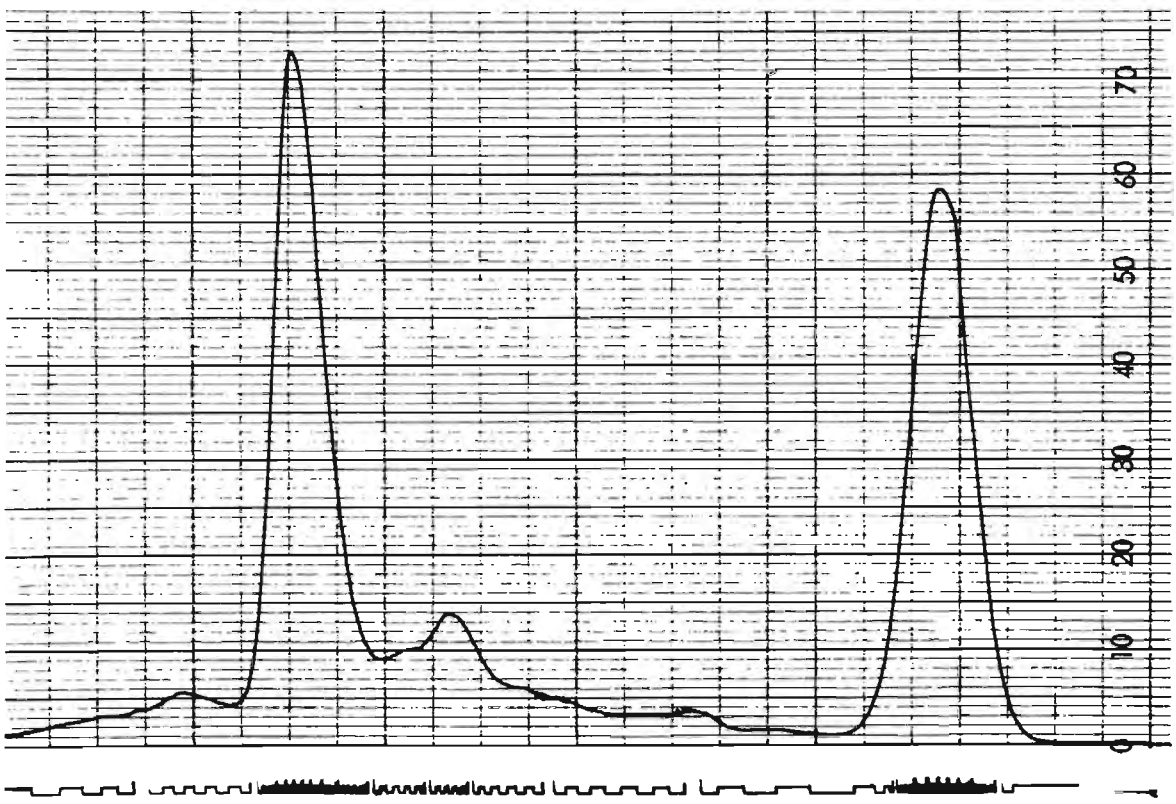

FIGURE 7: Electrophoretic pattern of plasma proteins of springbok no. 30, twenty-four hours after capture. Nothing has been added to this sample and note the similarity between Figures 6 and 7.

Cellulose acetate electrophoresis showed a distinctive pattern for both pure myoglobin and pure haemoglobin. When pure myoglobin and haemoglobin were mixed separately with plasma the same distinctive pattern could again be observed after electrophoresis of the mixtures (Figs 4, 5 and 6).

Electrophoresis was carried out on all the plasma samples of the springbok. In most of them a new peak appeared, which reached its highest level after 24 h (Fig. 7). This peak had the same appearance and position which was obtained with pure myoglobin. No conclusive evidence, however, could be obtained from the electrophoretograms with regard to the presence or absence of haemoglobin. We therefore decided to carry out poly-acrylamide gel electrophoresis on the following samples: pure myoglobin, pure haemoglobin and the plasma of stressed springbok, with and without the characteristic red colour. The results indicated that the plasma of stressed springbok showed an electrophoretic band which corresponded with that of pure myoglobin. It is important to note that both the coloured and normal coloured plasma showed this electrophoretic band.

Further investigation is, however, required before any definite conclusions can be drawn. For example, during cellulose acetate electrophoresis, fibrinogen also shows an electrophoretic peak in approximately the same position as myoglobin. Moreover, Garbus *et al.* (1964) state that the predominant muscle type lactic dehydrogenase isoenzyme (band 5), which increases with exercise, also migrates during cellulose electrophoresis with the gamma globulin fraction of plasma. In the present investigation, however, there was no correlation between individuals, which showed the highest L.D.H. level in the plasma, and the occurrence of this prominent new peak during electrophoresis.

The results obtained from acrylamide gel electrophoresis are, however, fairly convincing and point to the presence of myoglobin rather than haemoglobin. If we assume that the protein is myoglobin, it is also important to note that the presence of myoglobin in the plasma is not always accompanied by a red discolouration of the plasma. Whether the discolouration can be ascribed to the presence of a critical concentration of myoglobin in the plasma is not yet certain.

The correlation between myoglobin in the plasma and the elevation of the enzymes also needs further investigation. Schmidt and Schmidt (1969) stated that when myoglobinuria occurs the serum enzyme pattern approaches that of muscle tissue. The possibility that myoglobin in the plasma could be used as an index of the degree of stress and enzyme elevation in the plasma therefore appears promising, but at present is not nearly as practical as a measurement of plasma levels of C.P.K. Nevertheless, in the absence of facilities for chemical pathological investigations, the visual appraisal of the degree of brown discolouration of the plasma can, in our experience, be used as a practical clinical index of capture stress.

3.2 Experiment 2

Table 13 is a summary of all the plasma components which were measured in springbok shot from a concealed position without any previous disturbance or stress.

Although these animals fell in their tracks after being shot and respiration ceased almost immediately, the heart continued to beat for several minutes. Moreover, in most cases they displayed reflex kicking of the hind legs for about one to two minutes after being shot. Since all of them received clean headshots, the medulla oblongata was probably not immediately damaged by the shot and this may have allowed a severe sympathetic spasm to occur.

TABLE 13: Blood chemistry of springbok, shot without previous disturbance or stress.

DETERMINATION	MEAN	RANGE	N
HAEMATOCRIT	49,000	39,000– 54,000	8
BLOOD pH	7,121	7,051– 7,180	8
GLUCOSE (mg / 100 ml)	121,700	94,000–157,600	8
LACTATE (mg / 100 ml)	87,500	32,400–123,700	8
POTASSIUM (mEq / l)	7,700	4,800– 9,900	8
SODIUM (mEq / l)	153,000	149,000–157,000	8
C.P.K. (mU / ml)	28,000	24,000– 47,000	8
L.D.H. (mU / ml)	766,000	637,000–955,000	8
G.P.T. (mU / ml)	15,400	11,700– 19,100	8
ALDOLASE (mU / ml)	11,200	8,600– 18,000	8

From Table 13 it can be seen that only the plasma enzyme values were within normal limits. The elevated haematocrit and glucose values could possibly be ascribed to catecholamine release. The intensity and duration of this reaction was not the same in all cases and those animals which exhibited the highest glucose values also had the highest potassium concentration. This agrees with Ganong (1967) who states that plasma potassium rises coincidently with the glycogenolytic action of adrenalin.

Adrenalin also probably caused the elevation of lactate in the plasma and this increase was further enhanced by the intense kicking of the hind legs and the resultant anaerobic metabolism. The reduced blood pH may be a reflection of the effect of both the lactic acid and the accumulation of CO_2 after cessation of respiration.

There was no dramatic increase in the plasma sodium level but the increased potassium concentration may again indicate an increased membrane permeability, as suggested by Metivier (1969).

Although the real aim of this experiment, namely to establish normal values, could not be fully realised, it was still of interest to note the metabolic changes which occurred after the animals were considered to be dead.

4 SUMMARY AND CONCLUSIONS

This investigation encompassed a detailed study of the effects of capture and haloperidol therapy on various physiological and biochemical parameters in springbok. The results showed that capture in drop nets caused a marked increase in rectal temperature, cardiac rate and respiration rate. These changes were at first interpreted as being normal physiological adjustments to forced exercise, but subsequent blood analyses clearly showed that plasma levels of glucose, creatine kinase, lactate dehydrogenase and lactate were extremely high with a concurrent reduction in blood pH. It was concluded, therefore, that the capture operation had caused over-exertion of the animals, thus inducing anaerobic metabolism and a severe metabolic acidosis. Moreover, from concomitant studies of electrolyte flux it was observed that both metabolic acidosis and mechanical trauma resulted in hyperkalaemia which may, through cardiac arrest, be the ultimate cause of death. We are also of the opinion that the effects of over-exertion are dramatically potentiated by sympathetic stimulation as a result of an intense alarm reaction. The latter conclusion is based on the far milder reaction obtained in tame domestic animals (Gericke and Belonje, 1975).

Hofmeyr *et al.* (1973), Harthoorn and Young (1976) and Harthoorn and van der Walt (1974) have *inter alia* also demonstrated a metabolic acidosis, an increase in pulmonary arterial pressure and elevation of serum enzyme levels during capture operations on wild ungulates. On the basis of these findings Harthoorn, van der Walt and Young (1974) have advocated the use of $NaHCO_3$ infusions to overcome the metabolic acidosis. Although we agree with these findings, and although one of us (Gericke and Belonje, 1975) has been successful in controlling metabolic acidosis and hyperkalaemia in sheep with $NaHCO_3$ infusions, we feel that under conditions of mass capture operations in the field this treatment is impractical. Moreover, we wish to emphasise that the intense alarm reaction and resulting sympathetic response is largely responsible for potentiating the development of irreversible capture myopathy. Our studies have also provided evidence for the important effect of loss of K^+ from the muscle cells and the concomitant hyperkalaemia which occurs when an intense alarm reaction is evoked.

We feel, therefore, that in addition to the obvious precautions of avoiding over-exertion, hyperthermia and mechanical trauma during capture, every effort should be made to reduce the intensity of the alarm reaction. The most practical way of achieving this is through judicious use of tranquillising agents (Hofmeyr *et al.*, 1977). In the present investigation considerable success was achieved with the tranquilliser haloperidol (Searle). For example, the results show that, although haloperidol was not effective in blocking the elevation of serum enzymes, K^+ and the metabolic acidosis which occurred in response to the netting operation, the evidence suggests that it was able to markedly reduce lactate and glucose levels, indicating effective control of the alarm reaction. Moreover, after tranquillisation the blood picture gradually approached values usually considered normal for mammals until the 48 h and 120 h samples were taken without sedation, whereupon an immediate change occurred indicating that the alarm reaction had again set in. We are, therefore, of the opinion that haloperidol shows considerable promise in game capturing operations under a variety of practical conditions (*vide* Hofmeyr *et al.*, 1977); however, the absence of a true control group in the experimental design of this investigation, has not allowed us to make this conclusion with absolute certainty.

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