Incipient capture myopathy as revealed by blood chemistry of chased zebras

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I ABSTRACT

Zebras (*Equus zebra hartmannae* and *E. burchelli antiquorum*) were subjected to stress which gave rise to elevation of cardiac rate, haematrocit, plasma osmolality and plasma levels of glucose, lactate and creatine kinase. These changes were very similar to those observed in animals actually suffering from capture myopathy. It was therefore tentatively concluded that incipient signs of shock and therefore of capture myopathy were evident in the chased group of animals.

II INTRODUCTION

The recent increase in capture and transport operations involving large game has focused attention on a characteristic shock syndrome. This syndrome frequently leads to an irreversible condition which results in the loss of a large number of valuable animals. The syndrome is still poorly understood but occurs most frequently when animals are manhandled or restrained for several hours or more without judicial use of suitable tranquillisers or immobilising drugs. The initial signs are typical of severe stress but vary among individuals and there are marked differences between species in their susceptibility to the condition. Autopsy frequently reveals a definite myopathy of the skeletal muscles and in some cases myocardial involvement as well.

The syndrome has recently been called over-straining disease (Young & Bronkhorst 1971) and the marked involvement of both cardiac and skeletal muscle in the condition has led to the belief that over-exertion during capture and restraint depletes oxygen reserves. Then, under the influence of humoral factors brought into play by the alarm reaction, anaerobic metabolism and the resultant accumulation of anaerobic metabolites may contribute to complex metabolic changes which eventually lead to irreversible shock.

It is also our experience that the initial capturing technique has a profound effect on the survival of large game. For example, chasing animals to exhaustion before darting them with a suitable immobilising agent can lead to complications which are very difficult and expensive to treat. In contrast, if animals are darted after a minimum of alarm and disturbance, therapy, care and survival are usually routinely successful. For this reason then a study was undertaken to investigate the effect of alarm or fairly prolonged chasing upon the blood chemistry of zebras to discover if any of the presumed incipient signs of capture myopathy may develop.

III PROCEDURE

The investigation consisted of two phases. During the first phase an opportunity arose to collect blood samples during a routine cropping operation on eight mountain zebra (*Equus zebra hartmannae*). Careful

notes were kept on how far the animals ran and the degree of alarm they exhibited before being killed with a single shot. Within five minutes a blood sample was taken, immediately centrifuged in the field, and frozen for later analysis.

The second phase consisted of a planned experiment on the plains zebra (*Equus burchelli*) in the Etosha National Park. A total of ten animals was employed and five of these were exposed to stress by alarming them and chasing them at high speed in an open vehicle before darting them. The distance they were chased varied because of the problem of positioning the vehicle for firing the dart while travelling at high speed. The estimated average distance covered by the animals was, however, approximately 800 m at a full gallop and they usually continued to run for five minutes after darting. The remaining five animals were approached with extreme caution and darted with minimum disturbance from a closed vehicle. All animals were immobilised with a mixture of 3 mg etorphine hydrochloride/M99 (Reckitt) and 12,5 mg acetyl promazine (Boots) and the time taken for these drugs to take full effect varied from 4 min 8 s to 18 min 57 s.

Within five minutes after the animal became recumbant a heparinised blood sample was drawn from the external jugular vein, centrifuged in the field, and frozen for later analysis. Prior to centrifugation a sub-sample was taken for a micro-haematocrit determination. A second sample was collected one hour after the first and, as soon as possible after the animals became recumbant, rectal temperature, cardiac rate and respiration rate were recorded. These observations were again recorded one hour later and the animals remained immobilised during this one hour period. In certain cases it was necessary to administer additional M99 (0,5 to 1,0 mg) to maintain immobilisation.

Analysis of the plasma included determinations of plasma glucose, lactate, creatine kinase (CPK) and plasma osmolality according to the following methods:—

Glucose — using glucose oxidase and the method of Härtel, Fabel-Schulte, Lang and Rick (1968).

Osmolality — by freezing point depression using an automatic advanced research osmometer (Laboratory Instruments).

Creatine kinase — using the Boehringer activated CPK monotest and the method of Oliver (1955).

Lactate — method of Hohorst (1962).

Significance of statistical differences between treatment means and time of blood sampling were tested by applying Student's t test.

IV RESULTS AND DISCUSSION

The first phase of the investigation, during which mountain zebra were shot and careful notes were kept on the degree of alarm and exhaustion to

Plate 1. Capture myopathy: Mountain Zebra. Section of gluteus muscle showing an off-white area of degeneration and necrosis.

which the animals were exposed, could not reveal statistically significant results as the cropping operation was not statistically designed. Nevertheless, those animals which had been subjected to the greatest amount of stress showed a tendency towards exhibiting elevated creatine kinase, haematocrit and lactate levels in their plasma. During this period we were also afforded the opportunity of studying two mountain zebra which had been captured by conventional means using nets without the use of either immobilising drugs or tranquillisers and confined in wooden crates prior to shipment.

One of these animals, a stallion, began to show signs of extreme stress after two days and in spite of supportive therapy, consisting of cortisone, Catosal (Bayer), thiamine hydrochloride (Peterson), vitamin E and selenium (E-SE, S.A. Cyanamid), it died within 12 hours after treatment commenced.

The following post mortem results were obtained. The histopathological examinations in all the undermentioned cases were carried out by Dr P. A. Basson pathologist at Grootfontein, South West Africa. The autopsy revealed a variety of marked degenerative changes. Epicardial haemorrhages were present along the left coronary and longitudinal grooves and the histopathological examination of the myocardium revealed congestion, mild swelling and vesicularity of the fibres ,as well as patchy increased eosinophilia of other fibres. These changes were considered to be fairly terminal. The lungs showed moderate to severe congestion and oedema with concomitant emphysema. Froth was also present in bronchi and trachea. The bladder was extremely distended with coffee coloured urine and the kidneys showed mild nephrosis microscopically. The most marked change, however, occurred in the gluteal muscles. Gross examination of a cross section of these muscles revealed severe white streaking of the muscles similar to muscular dystrophy in domestic animals (Fig. 1). Histological examination confirmed that severe Zenker's degeneration and necrosis had occurred. Mild to moderate neutrophil infiltration was evident as well as very mild macrophage mobilisation and mineralisation of some necrotic fibres. The plasma levels of lactate and creatine kinase were elevated and, although the urine osmolality was greatly reduced, the concentration of inorganic phosphate in the urine was markedly increased.

In view of the above results the second phase of the investigation was carried out to discover if any incipient changes may occur during the initial stages of capture as a result of exhaustion, which could lead to, or were associated with an irreversible shock syndrome. The results were as follows.

1. Cardiac rate, rectal temperature and respiration rate

The effects of prolonged chasing upon the above parameters were exactly as expected, namely a highly significant ($P \leq 0,01$) increase in cardiac and respiration rate with a moderate but significant increase in rectal temperature (Table 1). These changes represent normal physiological adjustment to strenuous exercise but the marked degree of difference between the two treatment groups immediately after immobilisation suggests that the chased group was exposed to considerable stress, and confirms that the chasing had fulfilled its purpose. Of perhaps greater interest is the prolonged effect of chasing upon cardiac and respiration rate. For example, even one hour after immobilisation the mean respiration and cardiac rate did not decrease significantly. This result is, however, confounded by the fact that M99 has been shown to produce tachycardia in the equidae. In addition it depresses respiration and no definite conclusions can be drawn.

Also of interest is the fact that the rectal temperature in the standing group (not chased before darting) rose slightly ($P \leq 0,10$) during immobilisation. This may be due to the addition of acetyl promazine to the M99 as the former is known to disrupt normal thermoregulation in mammals (Pienaar, van Niekerk, Young & van Wyk 1966). Finally, from records of ambient temperature which were kept during the trial, it became evident that the degree of hyperthermia exhibited by the chased group was aggravated by high ambient temperature. Although this result was anticipated it serves to emphasize the important advantages of capturing game under cool conditions.

2. Haematocrit and plasma osmolality

The data pertaining to the effect of chasing upon haematocrit and plasma osmolality have been summarised in Table 2. The haematocrit values obtained immediately after immobilisation show a highly significant ($P \leq 0,01$) increase in the chased group when compared with the standing group. One hour later the values in the chased group had returned to normal and did not differ significantly from the standing group. Similarly, the osmolality of the plasma, immediately after immobilisation was significantly ($P \leq 0,01$) greater in the chased group and although they fell significantly during immobilisation the mean still differed ($P \leq 0,10$) from the standing group after one hour.

These results, together with those obtained for cardiac and respiration rate indicate that the chased group had been sufficiently alarmed and stressed to elicit autonomically controlled reflex reactions which are typically brought about by the catecholamines.

3. Plasma levels of glucose, lactate and creatine kinase

From the data contained in Table 3 it is clear that chasing caused a significant increase in plasma glucose and a very dramatic rise in plasma levels of creatine kinase and lactate.

Table 1. The effect of chasing upon rectal temperature, cardiac rate and respiration rate

	Chased group (n = 6)				Standing group (n = 5)			
	First reading[1]		Second reading[2]		First reading[1]		Second reading[2]	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Rectal temperature (°C)	40,04	± 0,39	40,42	± 0,66	38,73	± 0,49	39,6	± 0,89
Cardiac rate (counts/min.)	152,3	±44,5	135,6	±26,8	68,0	±13,4	81,3	±16,7
Respiration rate (counts/min.)	24,7	± 5,8	27,3	±11,6	13,7	± 4,6	19,0	± 6,4

[1] First reading taken immediately after immobilisation
[2] Second reading taken after one hour of immobilisation

Table 2. The effect of chasing upon haematocrit and plasma osmolality

	Chased group (n = 5)				Standing group (n = 5)			
	First sample[1]		Second sample[2]		First sample[1]		Second sample[2]	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Haematocrit (%)	50,3	± 3,4	43,6	± 2,5	42,7	±3,8	41,1	±5,1
Osmolality (mOsm/kg)	281,0	±10,3	263,0	±12,8	256,0	±6,0	251,0	±4,4

[1] First sample taken immediately after immobilisation
[2] Second sample taken after one hour of immobilisation

Table 3. The effect of chasing upon plasma levels of glucose, lactate and creatine kinase

	Chased group (n = 5)				Standing group (n = 5)			
	First sample[1]		Second sample[2]		First sample[1]		Second sample[2]	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Glucose (mg/100 ml)	141,0	±31,8	175,0	±26,1	83,0	±32,4	190,0	±19,1
Lactate (mg/100 ml)	68,9	± 6,4	47,3	±17,5	15,0	± 4,6	7,8	± 4,7
Creatine kinase (mU/ml)	77,0	±49,1	35,0	±25,2	19,0	± 7,2	17,0	± 4,6

[1] First sample taken immediately after immobilisation
[2] Second sample taken after one hour of immobilisation

The higher plasma glucose levels recorded for the chased group immediately after immobilisation can be ascribed to either increased catecholamine secretion, as described previously, or less likely to an accumulation of glucose as a result of depletion of oxygen reserves and a concomitant switch to anaerobic metabolism. The mean level of plasma glucose in the chased group, 141 ± 32 mg/100 ml is also far higher than the normal value of 102 ± 5 mg/100 ml established for horses by Evans (1971). The increased glucose values in the chased group do not agree with the results of Takagi and Sakurai (1971) who found a reduction in blood glucose in horses subjected to exercise. They do, however, agree with the findings of Axt, Richter and Ott (1968) who established that sudden over-exertion in horses caused a rise in blood sugar in the case of untrained animals whereas highly trained animals showed "good blood sugar adaptation". The marked rise recorded for the chased zebras would therefore seem to confirm that these animals were under considerable stress and their degree of fitness appears to be inferior to that of trained horses. The latter assumption may, however, not be valid because of the alarm the zebras were subjected to. Nevertheless, it is quite possible that trained horses could achieve superior physiological adjustment to vigorous exercise when compared to wild zebras, which seldom gallop for any great distance and only then, when they are threatened by predators.

Plasma glucose levels determined one hour after initial immobilisation show no significant change in the chased group and a highly significant ($P \leq 0,01$) rise in the standing group. At this stage these results can only be ascribed to the effect of the immobilising drugs until further research is carried out.

The very marked increase in plasma lactate levels recorded for the chased group immediately after immobilisation, indicates that anaerobic metabolism

was employed by this group of animals as a result of stress and the high demands placed on the oxygen reserves during their attempt to escape. The difference in mean values between the chased and standing group at this stage amounted to an increase of approximately 400% which, although high, is not excessively so when compared with values obtained for horses running 2 200 m by Takagi and Sakurai (1971). It is also evident from Table 3 that, after one hour of immobilisation, lactate levels remained relatively high in the chased group, whereas the mean values in the standing group decreased significantly ($P \leq 0,05$) by ± 100% over the same period. This suggests that the recycling of anaerobic metabolites and therefore recovery from the anaerobic condition may take a considerable time, even when animals have not been subjected to very severe stress.

One of the more interesting results of the investigation was the marked difference between the chased and standing group in respect to plasma levels of creatine kinase immediately after immobilisation. The mean level was approximately 400% higher in the chased group but one hour after immobilisation no statistically significant difference between the treatment means could be detected. Comparable results have been obtained in similar experiments. For example, McKechnie, Leary and Joubert (1967) found notable increases in serum enzymes in marathon athletes and attribute this result to changes in the permeability of muscle and other cell membranes associated with exertion. More specifically, Riethmüller and Wels (1972) have established that the mean creatine kinase activity in plasma in thoroughbred horses was 32 ± 4,5 mU/ml and in the case of animals with good performance this value rose by 30% during training. In the case of horses with poor performance, however, the resting value was increased threefold which compares well with the fourfold increase obtained in the zebras and they attribute this to pathological changes in the muscles.

Increased plasma levels of creatine kinase have also been recorded in the case of human patients suffering from muscular dystrophy (Dreyfus, Schapira & Demos, 1960a) and are greatly increased during myocardial infarction (Dreyfus, Schapira, Scebat, Resnais & Lenègre, 1960b). The specific cause for elevated levels of creatine kinase in the present investigation cannot as yet be explained with any certainty. It is, however, tempting to speculate that the alarm, intense muscular activity and anaerobic metabolism to which the animals were subjected, could have affected the integrity and therefore permeability of the muscle cell mebranes allowing this enzyme to enter the plasma in increased quantities.

V SUMMARY AND CONCLUSIONS

In final summary then it has been shown that, under the conditions of this investigation, even moderate stress in zebras leads to elevation of cardiac rate, haematocrit, plasma osmolality and plasma levels of glucose, lactate and creatine kinase. These results indicate that the alarm reaction and therefore increased catecholamine secretion was elicited in the stressed group of animals. Moreover, it would also seem that anaerobic metabolism was employed to a considerable extent by the chased animals and, judging by the persistence of lactate in the plasma, that this effect can be a prolonged phenomenon.

Finally, because of the similarity between the blood chemistry of the chased group of animals and the mountain zebra which died of shock, it is tempting to conclude that incipient signs of the shock syndrome, and therefore of capture myopathy, were already evident in the chased group of animals. At this stage this naturally cannot be done with any certainty.

Further research, employing a larger sample size and the measurement of a broader spectrum of metabolities, enzymes and electrolytes is required under various degrees of stress. The results are, however, sufficiently convincing to caution against undue alarm and exhaustion during the initial stages of game capturing operations.

VI ACKNOWLEDGEMENTS

We are grateful to Mr J. B. G. de la Bat, Director of Nature Conservation and Tourism, for making this study possible and to Dr S. S. Grovè of the South African Institute for Medical Research, Windhoek for providing some of the equipment. We wish to express our sincere thanks to Mr and Mrs A. Port of Valencia for their help and very generous hospitality during the initial stages of the project. Dr E. Joubert, Robin Gibbs and Joachim Lenssen are thanked for their help provided in innumerable ways.

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