Physiological Responses of the Buffalo *Syncerus caffer* Culled with Succinyldicholine and Hexamethonium

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Physiological responses of the buffalo *Syncerus caffer* culled with succinyldicholine (Scoline[1]) are reported and it is pointed out that a number of stressors are responsible for the observed changes in blood composition. The results are compared to those obtained in a pilot experiment from buffaloes culled with Scoline and hexamethonium, a ganglion blocker. Further investigation of the effects of hexamethonium may prove it to be of benefit to animals culled with Scoline.

Key words: Stress, succinyldicholine (Scoline), buffalo, hexamethonium, *Syncerus caffer*.

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Introduction

Changes in the blood composition of elephants and buffaloes culled in the Kruger National Park with the use of the muscle relaxant Scoline, have recently been investigated (Hattingh, Wright, De Vos, McNairn, Ganhao, Silove, Wolverson & Cornelius 1984). The values for blood obtained from undisturbed animals, killed by a brain shot, were compared with those from animals killed by several variants of the culling procedure. The results showed statistically significant differences in certain variables compared to control values and included plasma glucose, total lipid, lactate, cortisol and catecholamine concentrations and haematocit. The observed changes were regarded as physiological responses of herding and asphyxiation and it was pointed out that the combination of these two stressors is probably responsible for the stress of culling (Hattingh *et al.* 1984).

Subsequent studies have confirmed these results and have indicated that on average, brain death will occur within 11 min in buffaloes and within 17 min in elephants from the time Scoline has an effect on the respiratory muscles (and consequently blood gas tensions) but excluding the effects of exercise (Hattingh, Wright, De Vos, Ganhao, Silove, Knox, Ritchie, Bar-Noy, Cornelius & Fonseca 1985). By determining the critical levels of oxygen and carbon dioxide in blood which are necessary for consciousness it was also shown that

[1]Scoline (Glaxo)

elephants and buffaloes probably die because of the decreased Po_2 levels and not the increased Pco_2 levels when culled with Scoline (Hattingh, Cornelius, Ganhao & Fonseca 1986).

An examination of the above results points to the fact that the pituitary-adrenal and hypothalamo-sympathetic axes are activated during the culling procedure. Increased secretion of plasma catecholamines and cortisol are probably responsible for some of the other physiological responses observed in peripheral blood (Hattingh *et al.* 1984). The fact that no practical alternative to the use of Scoline is presently available (De Vos, Bengis & Coetzee 1983), possible ways of decreasing the stress of culling are required. One approach to this problem is to investigate ways and means of decreasing the magnitude of the physiological responses to the culling stressors and/or to decrease the time to death following darting with Scoline. This may be done by blocking the autonomic ganglia at the same time as blocking neuro-muscular transmission (Scoline effect). The present article reports the results of a pilot study conducted in the Kruger National Park on the effects of such ganglion blockade in buffaloes culled with Scoline.

Materials and Methods

Animals investigated were all part of the culling quota for 1987. They were killed in the usual fashion, i.e. herding by helicopter and darting with Scoline (De Vos *et al.* 1983), except for the experimental group (see below), the animals of which were killed with a combination of Scoline and hexamethonium.

Two experiments were performed:

1) For control values, jugular venous blood was obtained from 16 buffaloes (adult and immature males and females) as soon as practically possible after they had been culled. Each animal received 1 ml of a solution containing 18 g of Scoline in about 20 ml water (0,9 g/animal) between 6 and 24 min after herding commenced.
2) The experimental group consisted of six adult buffaloes individually herded in a fashion similar to the usual routine. They were then darted with a solution containing 0,9 g Scoline and 0,9 g hexamethonium (Sigma) between 4 and 7 min after herding commenced. Blood was obtained as before.

All samples were analysed for the variables reported (see below) according to standard techniques (Ganhao, Hattingh, Kay, Cornelius & Grobbelaar 1985). Results were compared statistically using Student's *t*-test and were regarded as significantly different at the 5% level.

Results

The buffaloes in the experimental group collapsed between 31 sec and 1 min 35 sec after darting (mean 57 sec). This mean time is about 21% shorter than the average given by De Vos *et al.* (1983) which is 72 sec. In addition the shortest time for a buffalo to collapse in the present study (31 sec) is shorter than the 36 sec recorded by De Vos *et al.* (1983).

The results for the blood analyses are shown in Tables 1 and 2. It is clear that haematocrit values were significantly higher in the experimental group and the cortisol, total catecholamines and glucose levels significantly lower than in the control group.

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Table 1
Individual and mean results for buffaloes killed with scoline

Buffalo	Haematocrit %	Total protein g/l	Total lipid g/l	Lactate mmol/l	Osmolality m0smol/l	Cortisol nmol/l	Total Catecholamines ng/ml	Glucose ng/ml
1	40	61,3	3,4	31,5	313	102	59,0	5,3
2	37	57,3	5,4	12,9	297	198	109,6	9,6
3	44	56,3	3,6	25,9	307	170	248,4	7,6
4	43	62,2	2,1	22,9	308	147	79,6	7,9
5	40	61,8	4,1	16,9	313	130	70,6	16,0
6	41	58,2	4,3	21,9	312	149	133,6	10,6
7	48	60,1	3,2	25,0	316	75	63,6	8,4
8	42	61,5	4,5	26,5	310	128	72,6	12,5
9	47	59,9	4,5	23,5	306	170	78,6	10,4
10	41	59,9	3,8	24,3	323	94	147,2	9,5
11	38	63,4	3,9	13,0	300	78	93,7	4,9
12	38	54,7	5,5	19,1	335	83	105,9	6,2
13	37	62,5	4,1	15,8	302	60	79,8	5,1
14	41	60,1	4,3	12,7	299	110	96,6	8,1
15	39	64,1	4,8	13,2	297	90	54,7	7,5
16	38	58,0	3,9	8,5	307	120	71,8	4,7
Mean ± S.D	41 ± 3	60,1 ± 2,6	4,1 ± 0,8	19,6 ± 6,5	309 ± 10	119 ± 40	97,8 ± 47,8	8,4 ± 3,0

Table 2
Individual and mean results for buffaloes killed with scoline and hexamethonium

Buffalo	Haematocrit %	Total protein g/l	Total lipid g/l	Lactate mmol/l	Osmolality m0smol/1	Cortisol nmol/l	Total Catecholamines ng/ml	Glucose ng/ml
1	41	61,8	4,5	14,1	330	10	42,1	4,3
2	44	60,8	4,5	15,4	293	54	59,6	5,7
3	44	62,0	4,8	14,7	323	29	–	4,5
4	47	61,8	3,8	17,4	344	33	33,8	4,2
5	46	63,4	5,2	20,9	331	10	94,5	4,5
6	50	62,5	2,9	12,9	299	100	30,5	5,4
Mean ± S.D	*45 ± 3	62,1 ± 0,9	4,3 ± 0,8	15,9 ± 2,9	320 ± 20	*39 ± 34	*52,1 ± 26,2	*4,8 ± 0,6

*Denotes-significantly different from the values in Table 1. ($P < 0,05$).

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Discussion

Data obtained from the experimental group in the present study are not directly comparable to those from the control group. Permission was obtained from the National Parks Board and the Department of Veterinary Services to conduct the pilot experiments with hexamethonium on a small group of animals provided the carcasses were left in the veld (see below). For practical reasons it was thus not possible to conduct the investigations on the same group of animals at the same time and the hexamethonium animals were herded, on average, for shorter periods of time than the control animals. The times used for the former group were, however, equivalent to the mean times for herding of buffaloes during the culling procedure as reported by De Vos *et al.* (1983).

Because of the above limitations, the results obtained should be interpreted with care. The significant differences reported between the results of the experimental and control animals do, however, indicate decreased activity in the hypothalamo-sympathetic and pituitary-adrenal axes of the former group. Scoline is a neuro-muscular blocking agent due to its affinity for nicotinic acetylcholine receptors in muscle. It has (very) limited ganglion blocking properties and animals exposed to this substance die because of asphyxiation with resultant increases in the concentrations of a number of variables usually regarded as physiological responses of stress (Hattingh *et al.* 1984). The results obtained in the present study for the concentrations of these blood constituents agree closely with those obtained previously on larger numbers of animals (Hattingh *et al.* 1984) and are thus an accurate indication of at least part of the physiological response to culling stressors.

Hexamethonium is an autonomic ganglion blocking agent due to its affinity for nicotinic acetylcholine receptors in ganglia. These nicotinic receptors are apparently different to those in muscle (Gilman, Goodman, Rall & Murad 1985). Hexamethonium crosses the blood-brain barrier in limited amounts and has some neuro-muscular blocking properties (Goldstein, Aronow & Kalman 1974).

The latter characteristic possibly explains why the animals in the experimental group collapsed sooner, on average, than those of the control group, i.e. a more effective neuro-muscular blockade was obtained. Because of the ganglion blocking properties, sympathetic discharge was decreased with resultant lower circulating concentrations of catecholamines (resulting in part in hypotension), cortisol and glucose. It is an important observation that these effects were obtained within the short time interval from darting to collapse of the animals (mean of 57 sec) and indicates that this substance is rapidly absorbed from the darting site. Hexamethonium will not kill by itself (observations on experimental animals in the laboratory, results to be published) but has the ability to decrease at least some of the physiological responses to culling stressors.

It is not generally appreciated that animals are exposed to a number of stressors when culled in the way done in the Kruger National Park. At least two of these, herding and Scoline, result in significant changes in blood composition (Hattingh *et al.* 1984). Because of the numbers which have to be culled each

year (De Vos *et al.* 1983), and bearing time constraints in mind, the animals are herded with a helicopter for variable distances. They are therefore forced to exercise and this is in itself stressful because they are not accustomed to running for any prolonged period of time. An analysis of results reported previously, which had been obtained from buffaloes that were shot after they had been running for variable distances (Hattingh *et al.* 1984), shows gradual increases in the concentrations of plasma glucose, total lipids, lactate, total protein, cortisol and total catecholamine concentrations and osmolality with time. Haematocrit values first show an increase which is followed by a decrease (haemodilution). This explains why the haematocrit values are higher in the experimental group than in the control group in the present study: the shorter time interval.

Animals to be culled, if they are herded, are therefore stressed even without the use of Scoline. Scoline administration by itself, without any associated herding or other disturbances, leads to similar changes in blood composition as does exercise (Hattingh *et al.* 1984). The effects of herding and Scoline, in isolation, on blood composition are, however, smaller than the cumulative effect of these two stressors during actual culling as indicated below. The only way presently available to kill an animal without it experiencing stress and still have the meat suitable for human consumption, is to shoot it through the brain without it being aware of the hunter. This is not practically possible in the Kruger National Park.

The above illustrates the multifactorial nature of the stressors in the culling procedure which causes the observed physiological responses (Table 1). Administration of hexamethonium simultaneously with Scoline, results in significantly decreased concentrations of a number of blood constituents (excluding haematocrit, see above). No objective measure of stress is available which may be used to determine whether the animals killed with hexamethonium were under less (or more) stress than those killed without it.

An objective assessment of the physiological responses to stressors has, however, been suggested which is based on the collective percentage change of the eight blood constituents used in the present study (Hattingh 1987). This measure, called the species specific experimental response to stressors (SSERTS) indicates that buffaloes culled with Scoline in the present study showed a 30% response and those killed with Scoline and hexamethonium a 22% response as opposed to 17% and 10% responses for herding alone and Scoline alone using data from our 1984 study. A decreased physiological response was thus present when hexamethonium was used in combination with Scoline. Furthermore, the animals collapsed sooner. Although some cardiovascular and respiratory activity was still present at this time, blood oxygen tensions were probably insufficient for continued consciousness (Hattingh *et al.* 1986). These two aspects, the decreased physiological response and the more rapid time to collapse after darting, strongly support the concept that the animals experienced less stress.

Finally, hexamethonium is used clinically in humans as an anti-hypertensive. It is non-habit forming, does not accumulate in the body and is excreted unchanged (Reynolds & Prasad 1982). The usual route of administration is by intravenous infusion. Doses of up to 3 g per patient are required on a daily

basis if the substance is to be given by mouth because of poor and erratic absorption in the gastro-intestinal tract (Harrington 1953). It is thus unlikely that any effect will result if the carcasses from animals killed with this substance and Scoline are used for human consumption. (No visual effects were observed in vultures and other scavengers who ate from the carcasses in the reserve). Although the results of the pilot study are positive, further work must be done on the distribution of hexamethonium in carcasses, the most effective w/w ratio with Scoline and the optimal volumes required to kill animals in the shortest possible time. The studies should also include elephants in order to see if similar effects are obtained. It is hoped that the inclusion of this substance in the culling procedure may eventually prove to be of benefit to the animals concerned.

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