Effects of etorphine and succinyldicholine on blood composition in elephant and buffalo

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Blood composition was investigated in anaesthetized elephant and buffalo and in elephant immobilized by succinyldicholine. Results obtained indicate that data from undisturbed animals shot in the brain closely approximate the situation in anaesthetized animals, except for blood-gas composition. Cortisol concentrations increase after anaesthetization and then decrease again, suggesting that the procedures involved are experienced to some extent as physiological stress. In the case of animals paralysed by succinyldicholine, cortisol and blood-gas results suggest that when this substance is used in culling procedures, elephant should be killed by a shot into the brain within 16 min after darting.

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Bloedsamestelling in genarkotiseerde olifante en buffels is ondersoek asook in olifante blootgestel aan suksinieldikolien. Die resultate toon aan dat data verkry van onversteurde diere wat in die brein geskiet is, goed ooreenstem met dié van genarkotiseerde diere, behalwe vir bloedgaswaardes. Kortisolwaardes neem toe na narkotisering en neem dan weer af, wat aantoon dat die betrokke prosedures tot 'n mate as fisiologiese spanning ondervind word. Kortisol- en bloedgasresultate verkry van olifante wat blootgestel is aan suksinieldikolien dui aan dat hierdie diere binne 16 min na pyling doodgeskiet behoort te word indien daar vir uitdunningsdoeleindes van hierdie middel gebruik gemaak word.

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Little information is available regarding the blood composition of elephant and buffalo and possible changes which may occur after the administration of anaesthetics and succinyldicholine (SDC). The latter substance is used extensively in game-culling procedures and interest has recently been revived following a report by Button, Bertschinger & Mülders (1981) that SDC-induced asphyxia led to catecholamine release by the adrenal medulla in conscious calves. By comparing pentobarbitone-anaesthetized sheep to conscious sheep, Button & Mülders (1983) also showed that lesser increases in arterial and central venous blood pressure, blood glucose and plasma catecholamines resulted after SDC administration in the anaesthetized animals. Hattingh, Wright, de Vos, McNairn, Ganhao, Silove, Wolverson & Cornelius (1984) attributed changes observed in a number of blood variables in culled elephant and buffalo to stress induced by a combination of herding and darting with SDC; control values being obtained from undisturbed animals shot in the brain. Because few baseline data are available for blood composition in elephant and buffalo, it is not known whether the results of Button *et al.* (1981) and Button & Mülders (1983) in sheep apply to other species and whether the control data used by Hattingh *et al.* (1984) reflect normal resting values. In the latter instance the results of Gericke, Hofmeyer & Louw (1978) are pertinent in that they found their control values obtained from individually shot springbok to be inadequate. Serial blood samples have now been obtained from elephant and buffalo at intervals up to 99 min after anaesthetization with etorphine (M99). In addition, individual undisturbed elephant were darted with SDC and serial blood samples obtained until the animal died. The results show that control data from shot elephant and buffalo (Hattingh *et al.* 1984) closely approximate those from anaesthetized animals.

Methods

Arterial and venous blood samples were obtained from animals in the Letaba area of the Kruger National Park during December 1983 and February 1984. When animals were to be anaesthetized with etorphine HCl (M99, Reckitt), individual buffalo and elephant were approached by vehicle and darted. Elephant received between 10 and 12 mg M99 and buffalo 7 mg M99 and 20 mg xylazine HCl (Rompun, Bayer). In the case of SDC, individual elephant were rapidly approached by helicopter, given four darts containing SDC (Scoline, Glaxo) and then left undisturbed. Each dart contained 7,5 ml SDC at a concentration of 56 g/100 ml water. In both procedures the disturbance involved was usually minimal, but some

animals were herded to some extent. As soon as the animal collapsed, blood sampling commenced and continued at regular intervals for up to 99 min in the case of anaesthetized animals (after which they were given an antidote) or until the animal died in the case of SDC. The latter group of animals formed part of those included in the current game-management programme of the Kruger National Park. Blood samples were obtained from ear arteries and veins (elephant) and ear arteries and external jugular vein (buffalo). Heart rate was measured by auscultation and respiratory rate determined by observation.

Heparinized arterial blood samples were collected anaerobically in glass syringes whose dead space was filled with heparin solution (5 000 U/ml Pularin). The samples were placed on ice and analysed within 2 to 3 h for pH, P_{O_2} and P_{CO_2} using a Radiometer PHM 71 analyser and BMS 3 MK2 Blood microsystem. Base excess was calculated from the Siggaard Andersen nomogram. The haematocrit was determined by centrifugation.

Heparinized venous blood (1 000 U/ml) was centrifuged immediately to obtain plasma which was stored on ice for transportation to the laboratory. Samples were divided into two aliquots, one of which was stored at −70 °C for up to six days. When all frozen samples had been collected, they were assayed for ACTH (ACTH immunoassay kit, Amersham, Code IM.66 and ACTH Double Antibody Liquid phase radio-immunoassay, catalogue number: KACD1, DPC), cortisol (Cortisol RIA kit, code IM 2021, Amersham and Cortisol Coat-A-Count solid phase radioimmunoassay, catalogue number: TKCO1, DPC), TSH (NHS-TSH RIA kit, catalogue number: KHTD1, DPC and TSH radioimmunoassay kit, catalogue number: 255319, Becton Dickinson and Co.), free T_3 (Free T_3 RIA kit, catalogue number: TKF31, DPC), total T_3(T_3 solid phase radioimmunoassay kit, catalogue number: 254312, Becton Dickinson and Co.) and growth hormone (Phadebas PRIST radioimmunoassay code 01-900-2-1406-2, Pharmacia Diagnostics). All samples were counted with an Auto In-V-Tron 4010 Automatic Gamma Scintillation counter.

The other portion of venous blood plasma was immediately analysed for blood-glucose concentration (GOD-Perid colorimetric test kit cat. no. 124028, Boehringer Mannheim), total protein (Biuret method using bovine serum albumin as standard) and total lipid (calorimetric test kit cat. no. 124303, Boehringer Mannheim). Protein electrophoresis was carried out using Gelman electrophoresis equipment and A/G ratios determined with a Beckman scanner equipped with an integrator.

Lactate concentration (enzymatic UV-method cat. no. 256773, Boehringer Mannheim) was determined using venous blood collected with fluoride/EDTA reagent (cat. no. 24710, Boehringer Mannheim) as anticoagulant. Body temperature was determined using a rectal temperature probe (Model B80/12, Bailey Instruments Inc.) inserted to a depth of 50 cm. A wet bulb, globe temperature Tempstress was used to monitor environmental conditions (Scitech Pty. Ltd.).

Not all analyses were performed on all samples. Results were compared statistically using the two-tailed Student's *t*-test and relationships determined by linear regression or polynomial regression by the method of least squares. Results are presented as means ± S.D.

Results

Anaesthetized animals

Both elephant and buffalo showed a statistically non-significant rise in rectal temperature between the first measurement and the last (Table 1) despite efforts to maintain constant body temperature by splashing water over the animals. During the same time interval, mean heart and respiratory rates decreased (significant, $P < 0,05$, in the case of elephant respiratory rate and buffalo heart rate). Table 2 shows the results of blood analyses. Except for plasma lactate and cortisol concentrations, none of the other variables investigated were time dependent. Also, no significant differences could be shown to exist in variables other than lactate and cortisol between animals which had been herded (exercised) to some extent and animals which were not disturbed by the darting procedure although blood pH and P_{O_2} values tended to be greater and P_{CO_2} values lower in the former group. (The blood-gas changes possibly result from hyperventilation due to exercise.) In undisturbed animals initial lactate values (first sample taken) were lower than those of animals which had been herded (significant for both elephant and buffalo, $P < 0,05$). Also, lactate values remained relatively constant during the period of observation in undisturbed animals, but decreased with time in the other group, as may be expected.

In the case of cortisol, initial values did not differ between undisturbed and herded animals of both species and the response in each case was the same. An example of cortisol

Table 1 General information relating to animals investigated

Procedure	Species	*N*	Time from darting[a] (min)	Sex	Shoulder height (m)	Chest circumference (m × 2)	Rectal temp.[b] (°C)	WB GT[c] (°C)	Heart rate[d] (min^{-1})	Respiratory rate[d] (min^{-1})
M99	Elephant	9	13,7 ± 4,9	4 Adult ♂ 4 Old ♂ 1 Young ♂	3,13 ± 0,23	2,09 ± 0,23	36,3 ± 0,6 to 37,2 ± 0,6	24,9 ± 2,7 to 25,6 ± 2,0	62 ± 12 to 51 ± 10	8 ± 2 to 4 ± 1
M99 Rompun	Buffalo	7	6,2 ± 1,8	4 Adult ♂ 1 Old ♂ 2 Young ♂	–	–	39,8 ± 1,1 to 40,1 ± 0,9	25,2 ± 2,4 to 26,0 ± 2,3	104 ± 28 to 54 ± 19	31 ± 11 to 21 ± 8
SDC	Elephant	4	10,3 ± 9,2	4 Adult ♂	3,33 ± 0,14	2,27 ± 0,09	–	–	37 ± 8 to 33 ± 10	9 ± 3 to 0

[a]From the time of darting until the animal collapsed. [b] Initial and final rectal temperature. [c] Initial and final wet bulb, globe temperature. [d] Initial and final heart rates and respiratory rates.

Table 2 Blood composition in anaesthetized elephants and buffalo

Variable	Elephant	Buffalo
pH	7,28 ± 0,08	7,37 ± 0,06
P_{CO_2} kPa (mmHg)	6,5 ± 0,8 (49 ± 6)	6,0 ± 1,1 (45 ± 8)
P_{O_2} kPa (mmHg)	7,9 ± 2,8 (59 ± 21)	7,6 ± 2,0 (57 ± 15)
Base excess ECF mmol/ℓ	−4,4 ± 3,3	0,2 ± 2,9
Glucose mmol/ℓ	4,9 ± 0,5	3,8 ± 1,6
Total lipid g/ℓ	2,35 ± 0,31	2,86 ± 0,36
Initial lactate mmol/ℓ [a]	1,3 ± 0,6 (undisturbed)	1,7 ± 0,7 (undisturbed)
	8,4 ± 3,0 (herded)	8,6 ± 4,2 (herded)
Free T_3 pmol/ℓ	4,3 ± 1,4	4,3 ± 1,1
Total T_3 nmol/ℓ	1,6 ± 0,2	1,2 ± 0,4
TSH μU/ml	2,0 ± 1,2	3,9 ± 1,7
Growth hormone m U/ℓ	0,42 ± 0,02	0,42 ± 0,03
Cortisol[a]		
initial nmol/ℓ	106 ± 43	60 ± 25
at peak nmol/ℓ	332 ± 96 (undisturbed)	63 ± 49 (undisturbed)
	493 ± 57 (herded)	100 ± 15 (herded)
response time between (min)	17,1 ± 5,5 and 29,7 ± 5,8	13,4 ± 2,4 and 28,4 ± 2,1
time at peak (min)	39,1 ± 7,1	28,4 ± 2,1
Haematocrit %	41 ± 2	31 ± 4

[a]See text for explanation.

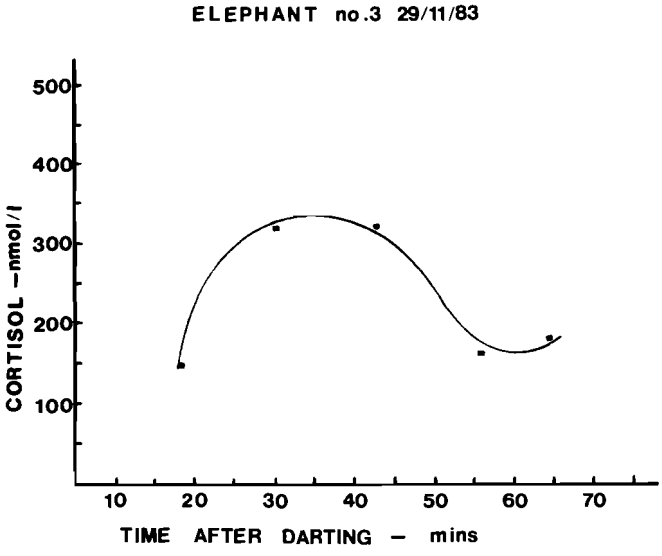

Figure 1 Cortisol response in elephant 3 upon anaesthetization. Third degree polynomial regression, $r = 0{,}9726$; $y = -821{,}1 + 92{,}9x - 2{,}3x^2 + 0{,}02x^3$.

response in an undisturbed elephant is shown in Figure 1. Cortisol values rose after the animal went down and then decreased again. Peak values were different in undisturbed and herded animals for both species, being significant ($P < 0{,}05$) in the case of elephant (Table 2). The times at which peak values were observed were not different for undisturbed and herded animals and are shown in Table 2. Response time for the increase in cortisol levels (time after darting) was judged to be after the first sample and before significantly increased values for this variable (compared to the first sample) were recorded (Table 2).

In one elephant cortisol levels continued to increase for up to 70 min after darting (when the animal was given the antidote). Elephants usually collapse on their sides but this one went down on his brisket. Respiration was laboured and heart rate decreased from an initial value of 55 to 25 beats/min 42 min after darting. At this stage he was pulled over to his side. Respiration was then easier and heart rate increased to 44 beats/min.

Animals darted with scoline

The results obtained are shown in Tables 1 and 3. Mean heart rate decreased and was variable just before the animal died. Respiration decreased to zero before the heart failed. Certain variables in blood did not change with time and others did. In the latter case, individual variation (related to time) was pronounced. The change with time per individual was calculated (linear regression) and the mean values for the four animals are shown in Table 3 in addition to the mean initial values. It is clear that total protein, haematocrit, total lipid, TSH and free T_3 did not change with time. All the other variables investigated were time dependent and the magnitudes of change were similar for the different animals. In the case of cortisol the mean time before a significant increase was noted was between 7 and 16 min. The relationship between ACTH and cortisol concentrations was significant at $P < 0{,}05$ (linear regression, $y = 1{,}8x - 159{,}2$; $r = 0{,}907$).

The relationship between P_{CO_2} and P_{O_2} for both series of experiments (SDC and M99) is shown in Figure 2. The correlation coefficient for the linear regression line was $r = -0{,}8122$ and for the second degree polynomial regression curve $r = -0{,}8380$, both being significant at $P < 0{,}05$. It is clear that the curve is a better fit for the data obtained.

Discussion

Previous studies on elephant blood are summarized and discussed by Moore & Sikes (1967), White & Brown (1978), Brown, White & Malpas (1978) and Brown & White (1979). Most of these deal with general haematology and in many cases blood was obtained from shot animals. No reports could be found in the literature dealing with serial sampling in anaesthetized animals. In the case of wild buffalo, no results are available in the literature.

The possibility of seasonal variation in elephant blood composition has been pointed out by Brown & White (1979) and

Table 3 Blood composition in elephants darted with SDC

Variable	Value and change per minute[a]
Initial glucose mmol/ℓ	4,9 ± 0,8; 0,11 ± 0,07 mmol/ℓ/min increase
Total lipid g/ℓ	4,23 ± 0,55
Total protein g/ℓ	8,4 ± 0,8
Haematocrit %	43 ± 1
Free T_3 pmol/ℓ	1,7 ± 0,4
Initial lactate mmol/ℓ	10,8 ± 2,3; 0,68 ± 0,59 mmol/ℓ/min increase
Initial growth hormone mU/ℓ	0,29 ± 0,10; 0,008 ± 0,001 mU/ℓ/min increase
TSH μU/ml	3,21 ± 0,76
Initial ACTH μIU/ml	148 ± 88; 7,1 ± 2,7 μIU/ml/min increase
Individual ACTH response time	No effect for 4,5 – 12 min
Initial cortisol nmol/ℓ	146,0 ± 49,3; 11,6 ± 6,7 nmol/ℓ/min increase
Individual cortisol response time	No effect for 7 – 16 min
Initial P_{O_2} kPa (mmHg)	4,80 ± 0,80; 0,41 ± 0,07 kPa/min decrease (36 ± 6; 3,1 ± 0,5 mmHg/min decrease)
Initial P_{CO_2} kPa (mmHg)	9,33 ± 1,60; 0,52 ± 0,15 kPa/min increase (70 ± 12; 3,9 ± 1,1 mmHg/min increase)
Initial pH	6,93 ± 0,11; 0,02 ± 0,00/min decrease
Initial base excess ECF mmol/ℓ	– 16,6 ± 5,1; 0,15 ± 0,08 mmol/ℓ/min decrease

[a]See text for explanation

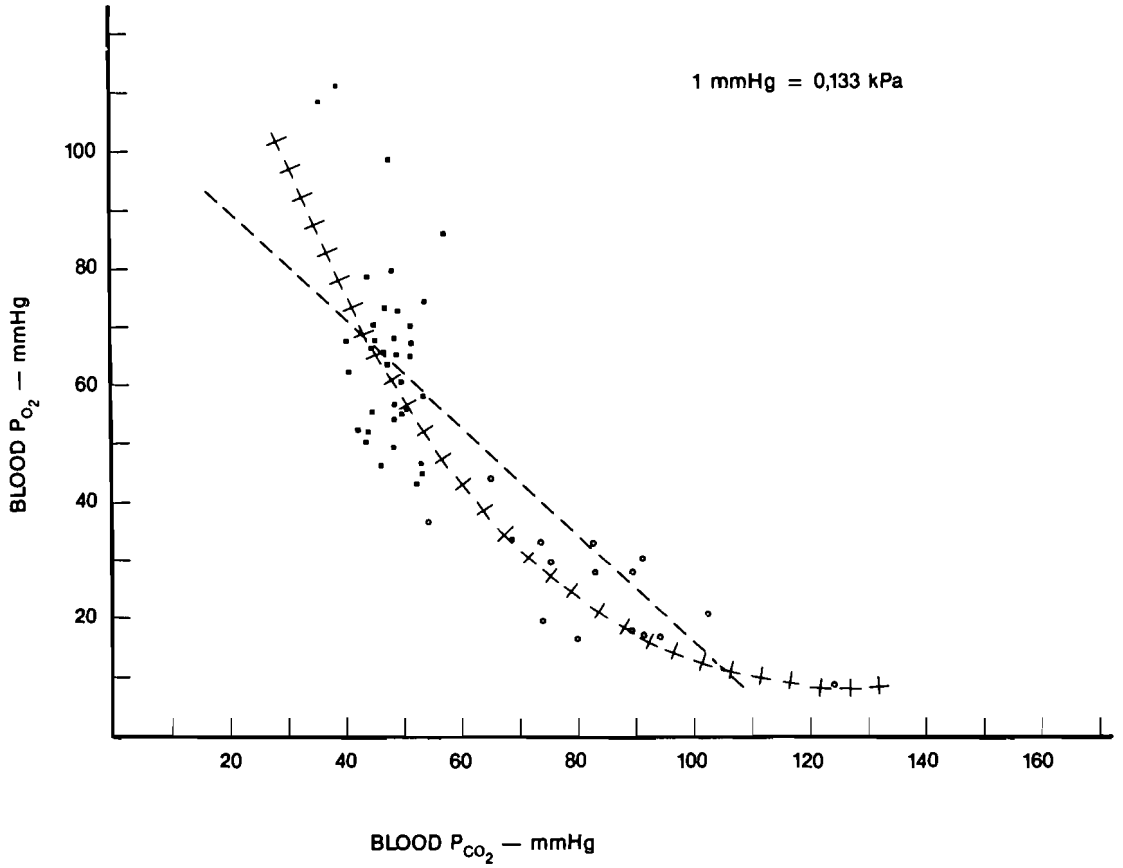

Figure 2 Relationship between blood P_{CO_2} and P_{O_2} for elephant anaesthetized with M99 (▪) and darted with SDC (o). The dashes (---) represent the linear regression line $y = -0{,}94x + 108{,}77$ and the crosses (+ + +) the second degree polynomial regression curve fitted to the data, $y = 161{,}9 - 2{,}6x + 0{,}01x^2$.

Moore & Sikes (1967). In addition, geographical and other factors are also possibly of importance. The animals used in the present study were obtained from the same geographical region and during the same season as those used in a previous study (Hattingh *et al.* 1984). As pointed out in the latter report, our values for plasma proteins, electrolytes and lipids from animals shot in the brain were similar to published data. Now, however, we are also in a position to compare data obtained from undisturbed animals shot in the brain with data from anaesthetized animals (this report and Hattingh *et al.* 1984). In the case of both buffalo and elephant, mean blood pH and P_{O_2} values were higher in the present study and P_{CO_2} values were lower

(significant for P_{O_2} and P_{CO_2} in buffalo, $P<0,05$). This is not surprising, seeing that in the case of shot animals, elephant blood could only be obtained after 45 s and in buffalo, after the carotid artery and jugular vein had been exposed. By accepted human limits of arterial pH 7,38 – 7,42, P_{CO_2} of 5,5 – 6,1 kPa (41 – 46 mmHg) and P_{O_2} of 11,3 – 12,7 kPa (85 – 95 mmHg) at sea-level, anaesthetized elephant and buffalo exhibited hypercapnia, acidaemia with a small base excess or a base deficit and hypoxia. This may be attributable to the effects of anaesthesia and postural embarrassment of respiration. However, it is apparent from Figure 2 that in elephant, respiration restricts the P_{CO_2} variation whilst P_{O_2} levels will ensure oxygen saturation in excess of 80% (Riegel *et al.* 1967).

Apart from the items pointed out above, no other statistically significant differences were evident between the present results on anaesthetized and darted animals and those for shot animals (Hattingh *et al.* 1984). This also applies to the initial lactate and cortisol values. The lactate values for herded animals reported here (Table 2) again agree closely with those previously found for herded shot animals (Hattingh *et al.* 1984). The only other result on cortisol levels in elephants is that of Brown & White (1979), with a value of 315 ± 182 nmol/ℓ. The present results show smaller variation and indicate that response time is between 17 and 30 min after darting, agreeing with a report by Thurley & McNatty (1973), that several minutes elapse before cortisol levels commence to rise in ewes after exposure to stress. The fact that both buffalo and elephant experience anaesthetization as some form of stress is shown by the observation that cortisol concentrations rise after this procedure and then subsequently return to lower levels. Individual variation is evident and the degree of herding before anaesthetization and body position after collapse seem to be complicating factors. It may thus be concluded that, apart from blood-gas composition, data obtained from undisturbed animals shot in the brain closely approximate data from anaesthetized elephant and buffalo. How close these results are to the normal, undisturbed and resting situation can only be determined by remote sampling procedures.

We have previously reported that total lipid and protein, TSH and T_3 concentrations did not change with time in the interval until death after undisturbed elephants were paralysed by SDC (Hattingh *et al.* 1984). The present results confirm this and in addition provide the magnitudes of change per unit time for a number of variables until death. Animals varied in their response relative to time and this was possibly due to differences in the effectiveness of the darting procedure (whether the darts hit bone or soft tissue), speed of absorption of SDC, degree of herding, etc. However, once individual variation had been eliminated by calculation, the results were very similar for all animals. Of importance is that cortisol concentration did not change for 7 to 16 min after darting. Also, blood P_{O_2} decreased on average by 0,41 kPa/min (3,1 mmHg/min) and did not change suddenly from normoxic to hypoxic. Assuming a normal P_{O_2} of 7,9 kPa (59 mmHg) in arterial blood (Table 2, M99 experiment), and a half saturation oxygen tension of 2,9 kPa (22 mmHg) (Riegel, Bartels, Buss, Wright, Kleihauer, Luck, Parer & Metcalfe 1967), it would require about 16 min (from the time SDC exerts its action) for the blood to be 15% saturated with oxygen. The changes in pH and P_{CO_2} (and possible increased 2,3 diphosphoglycerate levels mediated by cortisol increase), would all tend to increase the availability of blood oxygen. It is not known at what P_{O_2} level an elephant would lose consciousness as brain function will also be influenced by blood flow. However, while consciousness lasts it must be presumed that decreasing oxygen saturation combined with rising P_{CO_2} levels will cause severe discomfort and stress. The present results therefore indicate that on average, elephants exposed to SDC should be killed by a shot in the brain as soon as possible after darting and certainly not later than 16 min (considering our cortisol and P_{O_2} results). This is indeed the case in the culling operations in the Kruger National Park.

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