PHYSIOLOGY

OF

CAPTURE MYOPATHY

BY

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Frontispiece. The tragedy of conflicting perfections!

This thesis

is

dedicated to

THE PERFECT LIVING MASTER

SADAR CHARAN SINGHJI MAHARAJ

with

love, gratitude and devotion

Today there is a wide measure of agreement, which on the physical side of science approaches almost to unanimity, that the stream of knowledge is heading towards a nonmechanical reality; the universe begins to look more like a great thought than like a great machine. Mind no longer appears as an accidental intruder into the realm of matter; we are beginning to suspect that we ought rather to hail it as the creator and governor of the realm of matter....

- Sir James Jeans

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ABSTRACT

THIS investigation has been carried out in three nature reserves in the Transvaal, the Kruger National Park and in two game reserves in Natal. Specimens for trace element analysis were collected also from Natal and from three Lowveld nature reserve complexes.

A study of 104 individuals of nine species of animals

after locomotory stress indicates that the primary cause of mortality after a short (2 km) chase is a profound acidaemia, with muscle pH *significantly* lower than that of central venous blood. There was a *significant* correlation between pH and blood lactate.

Systemic changes include aberrations in the electrocardiogram configuration associated with hyperkalaemia, depression of calcium ions, tachycardia (up to 350 beats per minute), hypotension and a pulmonary arterial hypertension.

High capillary haematocrits together with low PO_2, pH and high PCO_2 indicated peripheral stasis. A rise in the haematocrit of central blood after restoration of the blood pH by infusion of bicarbonate indicated a return of sequestrated blood to the circulation.

Plasma myoglobin and haemoglobin showed a *significant* correlation with distance run. There was a *significant* rise in blood potassium to near lethal levels.

Reduced kidney function due to vasospasm, relieved by alpha-blockade with phenoxybenzamine hydrochloride, is believed to predispose to tubular blockade with blood and muscle pigments as demonstrated at autopsy.

High levels of GOT, GPT, CPK and LDH were seen in all animals subjected to locomotory stress (except in trained animals).

There was a *significant* difference between stress due to transport and exercise stress.

Investigation of liver content of trace elements on a seasonal basis revealed a *significant* correlation between chlorine, cobalt, magnesium, sodium and zinc. Major differences were determined exceeding 200 percent in the seasonal variation of selenium as well as copper, cobalt and other essential trace elements.

Highly significant differences were apparent in enzyme peaks between the first and subsequent runs. Differences were also seen in plasma haemoglobin and myoglobin between animals run for the first time, and after several dummy or training runs at low speed.

It is concluded that preliminary exercising of animals and their familiarisation with capture methods and corrals is the best prophylactic against stress during capture.

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CONTENTS

PAGE

PAGE

CHAPTER FOUR: ACID-BASE BALANCE AND BLOOD GASES

CHAPTER FIVE: CARDIAC CHANGES

CHAPTER SIX: BLOOD PRESSURE

PAGE

PAGE

PAGE

CHAPTER FIFTEEN: THERAPY

CHAPTER SIXTEEN: GENERAL DISCUSSION

PAGE

TABLES

FIGURES

PAGE

PAGE

PAGE

PAGE

PLATES

APPENDICES

CHAPTER ONE

GENERAL INTRODUCTION

INTRODUCTION

THE object of this study is an investigation into the physiological mechanisms underlying the condition of capture myopathy, a lethal condition exhibited by wild animals, mainly after mechanical capture and chasing.

The condition is already well known from the lesions seen at autopsy. These were first described in Kenya from a specimen of the rare Hunter's antelope *Damaliscus hunteri* that had died during an attempt to relocate some of these animals to the Tsavo National Park (see below). Numerous *post mortem* examinations were subsequently made by investigators in South Africa and the lesions have been described recently in considerable detail by Basson and Hofmeyr (1973).

The field of investigation has, perhaps inevitably, broadened considerably. The assignment was essentially practical in that the ultimate object was to find a therapy for the myopathy condition. It soon became evident that the underlying causes of deaths in captured wild animals must be sought not only in the method of capture and the resulting physiological derangements, but also in (a) the

nutritive state of the animals before capture and in (b) the post capture treatment and conditions of captivity. The first of these lent itself to straightforward scientific study and has consequently been the subject of a considerable amount of investigation during the course of this work especially with regard to absolute and seasonal shortages of trace elements. The latter (b) was found to include essentially sociological aspects making it unsuitable as other than a whole-time specifically authorised study. From such figures as are available it would appear that wild animal deaths during the long-term holding in quarantine pens exceed those of capture as it is now being carried out (during 1975) in the Transvaal Division of Nature Conservation.

The central field of research, that of the underlying physiological changes that lead to capture myopathy, was also subject to a degree of re-orientation. It was soon discovered that a great deal of the changes that occurred during capture, and particularly the degree of these changes, was due to largely unnecessary and mostly unwarranted stress put on the animal during the chase. During observations in the early stages of this work it was established that the most common causes of death in animals such as springbok *Antidorcas marsupialis* captured in nets were fractures of legs, neck and back, and luxations particularly in the lumbo-sacral region. It has been observed (S.A. Veterinary Congress 1973), bearing in mind the manner in which animals are captured, that far from being surprising that

animals died after chasing, it is a wonder that any survive!

It was established during the early part of the investigations, that animals captured in the usual way by chasing with trucks exhibited degrees of acidosis considerably exceeding those of human athletes and also horses during competitive racing. This is perhaps not unexpected as man and horse run in a competitive spirit with relatively little coercion while wild animals run to maximum capacity in an all out effort to escape. Adult specimens of wild animals such as zebra *Equus burchelli* and wildebeest *Connochaetes taurinus* will run during attempts to catch them by hand from a truck, until they die; the mechanism of death being primarily a profound acidosis.

The comparison with human athletes and horses is further falacious in that these are trained, and wild animals may be regarded, in this context, as being grossly 'out of condition'. One of the more interesting findings, from a practical aspect, during this work is the rapidity with which wild ungulates adapt to exercise and the marked increase in resistance to capture stress that occurs in the animals after only a few mock runs. These changes are measurable in the reduced rise in the level of enzymes, such as creatinine phosphokinase, but particularly also in the reduced discolouration of the blood plasma by myoglobin and haemoglobin, as well as by the less objective criteria of clinical condition. Animals such as black

wildebeest *Connochaetes gnou* showed striking adaptation when subjected to mock capture runs during which they became trained physically, accustomed to the capture procedure, and gave the impression of reduced fear. As a result, the clinical signs of stress and exertion were greatly reduced and the whole capture operation faciliated.

PREVENTION

THE prevention of capture stress and the ensuing myopathy is one of the more eminently practical considerations to emerge from this study. Whereas the restoration of a normal range of blood pH by infusion with bicarbonate, the consequent reduction by dilution of hyperkalaemia and so forth, has reduced losses both on an experimental and operative basis, the method is cumbersome to use, entails extra and possibly excessive handling, and beyond the capacity of most animal trappers. Conversely, the method whereby the animals are subjected to a series of dummy runs through the wing (only) of a capture corral appears readily practicable under most, if not all, the conditions under which animals are captured in the Transvaal. Similarly on our exercise track, it was found that the various groups of animals could be used only once or twice before they ceased to exhibit stress symptoms in spite of running at the same speed as on previous experiments.

This work has derived both advantage and disadvantage from its broad terms of reference, and similarly advantage and

disadvantage from standing on the sidelines of a large organisation concerned with the breeding and relocation of a number of animals comprising fifteen species from seven reserves. The earlier capture team tended to negate the myopathy problem, kept scanty records of losses and declined to collect necropsy specimens. It furthermore proved unfeasible to examine animals captured under routine capture conditions so that the research programme was switched entirely to an experimental basis using small groups of animals whenever these became available. The first experimental work was performed in October 1972 when 18 blesbok *Damaliscus dorcas phillipsi* had to be evacuated from Percy Fyfe Nature Reserve. Square-lipped rhinoceros *Ceratotherium simum* were examined in Natal during a week's investigation of unexpected transit deaths. Tsessebe *Damaliscus lunatus*, when a half-dozen bulls were to be disposed of and similarly nine excess sable bulls *Hippotragus niger* were recently made available at Hans Merensky Nature Reserve. The lack of substantial groups of animals from any particular species has had little impact on the physiological work while the variety of species has been a stimulus both to the investigation and to adapt our methods and apparatus from animal to animal in a variety of sizes from blesbok to sub-adult eland *Taurotragus oryx*. It has, however, retarded the work on therapy which depends for results largely on the statistical evaluation of parameters and therefore requires a reasonable number (arbitrarily set at a minimum of 10) of similarly-sized animals in comparable condition.

In the course of an effective period slightly exceeding three years it has proved possible to carry out nearly 60 000 tests on live animals comprising eight species. These include 18 blesbok (6 768 tests) a group of 25 zebra and wildebeest in the Kruger National Park (31 998 tests) and ten eland (15 694 tests), or 23 660 tests during 1972 and 1973, 18 252 tests during 1974 and 18 064 tests during 1975. These studies, performed almost entirely in the field or in temporary laboratories, averaged 600 tests per animal, i.e. 104 animals from which 4 491 samples were taken (Table 1). In addition, 253 liver samples for trace element work were provided from animals culled for various reasons unconnected with this work, in Zululand, the eastern Transvaal, and S.A. Lombard Nature Reserve; no animals have been killed specifically for the purpose of these experiments. Apart from a limited series subjected to acid digest, and spectrofluorimetry for selenium, the analyses for 17 trace elements (all but the drying, maceration and cataloging) have been performed by the Atomic Energy Board. Finally, 44 autopsies have been performed.

NOMENCLATURE

AT this present time there are almost as many names for the capture myopathy syndrome as published papers. It is therefore necessary at this early stage to gain a clear concept of the condition being investigated, and to define

Table 1: Total parameters and numbers of tests carried out on all live species investigated.

	acid-base	enzymes	clin. param.	plasma protein	porphins	body meas	parasites	minerals	metabolites	cardiac param	totals
blesbok	6 024	864	270	-	432	23	-	-	-	-	7 613
tsessebe	318	1 872	231	-	-	66	12	-	576	6	3 081
zebra	15 801	2 632	1 000	-	-	17	-	-	-	652	20 102
wildebeest	9 024	1 504	730	-	-	14	-	-	-	624	11 896
nyala	192	32	-	-	-	2	-	-	-	-	226
rhinoceros	56	70	-	210	-	7	21	56	14	-	434
sable	-	32	354	-	4	9	9	36	372	114	930
eland	10	6 216	1 647	-	-	10	-	-	7 770	41	15 694
totals	31 425	13 222	4 232	210	436	148	42	92	8 732	1 437	59 976

it in relation to the terminology it is proposed to use. It is important also, to ascertain if the various reports on deaths due to capture are in fact related to the same syndrome

The term 'white muscle disease' seems unduly confusing in that it has already been used for the condition that results on deprivation of the selenium/vitamin E complex. The same criterion applies to allied terms such as 'fish muscle disease', 'vlekspiersiekte' and so forth. 'Pale musole disease' is a term used to describe a condition in carcase meat which occurs when the body pH falls excessively after slaughter. Associated with this condition is the problem of 'pale, soft, exudative meat', and although this has certain manifestations in common with capture myopathy, the term is best restricted to the specific phenomenon in the pork industry, and seems to be influenced by the breed of pig, method of slaughter and the type of feed

'Spastic paresis' is a term used for a congenital condition in calves, and this therefore has the same disadvantages, although a congenital predisposition to death from capture stress is possible and awaits investigation.

The various terms that have been used appear to relate to the same syndrome in that they refer to a condition associated with animals dying after either capture or other forms of exhaustion and exhibit muscle degeneration on *post*

mortem examination.

Lesions other than the muscle lesions themselves tend to be indefinite and related to shock and those expected from hypotension and death. The term 'myopathy' therefore seems valid and 'capture myopathy' links the clinical and the autopsy picture to that of animal capture. 'Overstraining disease' is possibly a close contender. It suffers from the disadvantage that the term 'disease' tends to have a less than acute correlation, and because some doubts have recently been cast of the role of 'overstraining' as a factor. If death can indeed result on either massive anaerobic glycolysis or from nervous muscle tension, the term 'overstraining' has a misleading connotation associated with the dubious concept of disease due to long chases at undetermined speeds. The term 'disease' is further inappropriate in view of the broad spectrum of symptoms exhibited by captured animals, and the different manifestations that occur as a result of the varying methods used. For this reason the descriptive term 'syndrome' may be more appropriate. Whereas inevitably the stress of capture tends to precipitate bacterial and other diseases in captured wild animals (in Kenya it was noted that captured black rhinoceros *Diceros bicornis* from so-called 'fly' areas frequently succumbed to trypanosomiasis unless treated prophylactically on capture - own records), it seems preferential to separate the clinical entities of capture myopathy and possible intercurrent infections or enzootic or epizootic diseases that may occur during quarantine or holding.

The clinical and *post mortem* picture described in the various reports on this syndrome bear close comparison. These date back a decade when the pathological picture in animals that died following mechanical capture was described by Jarrett, Jennings, Murray and Harthoorn (1964) from a specimen of the wild Hunter's antelope. The examination of pale areas in the skeletal muscle showed hyaline degeneration with loss of cross striation. Some muscle fibres showed transverse breaks and some wholly degenerated with marked proliferation of the sarcolemmal sheaths giving rise to a bizarre cellular appearance. The condition was described as 'muscular dystrophy' and stated to be indistinguishable histologically from 'white muscle disease' as seen in cattle suffering from vitamin E deficiency from poor feeding or from overfeeding certain oxidant 'anti-vitamin E' substances.

A similar condition has been referred to as 'muscle necrosis' by Young (1966) in red hartebeest *Alcelaphus buselaphus* describing lesions of discoloured muscle fibres in the heart and skeletal muscle, as well as degenerative changes in liver and kidneys. The principal clinical sign was paralysis, although seven other animals died suddenly without manifesting clinical signs.

A condition of *leg paralysis* was reported in the greater and in the lesser flamingo *Phoenicopterus ruber roseus* and *Phoeniconaias minor* by Young (1967). Here also the skeletal muscle fibres showed loss of their striated appearance and were

changed into a homogeneous mass together with rupture of the individual fibres. Necrotic foci were found in the myocardium.

Young and Bronkhorst (1971) refer to the condition as 'overstraining disease' in game stating that the clinical signs usually appear at any time from a few hours to one to two weeks after excessive exercise. They describe degenerative muscle lesions and compare the degeneration to 'paralytic myoglobinuria' in the horse. The apparently identical condition has been described by Ebedes (1969) as occurring in oryx *Oryx gazella gazella* and in a number of wild animals by Basson, McCully, Kruger, van Niekerk, Young, de Vos, Keep and Ebedes in 1971. Basson and Hofmeyr (1973) describe the condition as 'capture myopathy' and report that it affected a number of antelope, including the red hartebeest, springbok, eland, oryx, roan antelope *Hippotragus equinus*, sable antelope, kudu *Tragelaphus strepsiceros* and nyala *Tragelaphus angasi*, as well as Burchell's zebra, mountain zebra *Equus zebra hartmannae*, giraffe *Giraffa camelopardalis*, buffalo *Syncerus caffer*, the black rhinoceros and even primates and birds. They state that the condition has no seasonal incidence. Deaths up to one month after capture in zebra were noted as a result of fibrotic lesions in the myocardium. They report that myocardial lesions are found in elephant *Loxodonta africana* immobilised with succinylcholine chloride after chasing, 30 percent of the carcase meat being affected.

The condition was described as occurring in baboons *Papio ursinus*, the young being more prone to the condition than large dominant males, by McConnell, Basson, de Vos, Kuntz and van Niekerk (1972). They give clinical symptoms as pain, stiffness, and improper functioning of certain muscles leading to paresis, torticollis, prostration and paralysis, laboured respiration and tachycardia with sporadic myoglobinuria. The lesions are described as lighter grey-ish brown often striated with haemorrhages due to rupture in skeletal and heart muscle. Histological examinations show areas of degeneration and necrosis, mineralisation of necrotic fibres, sarcolemmal proliferation and fibrosis as in white muscle disease of domestic animals. Lesions are described as occurring in kidney, adrenals, liver, spleen, lymph nodes and lung. The condition is compared to 'transport myopathy' in cattle transported over long distances under crowded conditions, and to 'paroxysmal paralytic myoglobinuria' of man as described by Scibold, Roberts and Wolf (1971). 'White muscle disease' occurs in live trapped mountain goats *Oreamnos americanus* and has been described by Hebert and Cowan (1971). Serum glutamic oxaloacetic transaminase (GOT) values and the histological changes in the muscle tissue of the hind leg as well as the symptomatology were all stated to be compatible with the diagnosis of factors such as 'selenium deficiency myopathy' or 'white muscle disease'. The muscle showed patchy degenerative changes of either hyaline or granular nature which were apparently consistent with those of 'white muscle nutritional myopathy' in cattle and sheep.

Other terms used for the capture myopathy syndrome are 'capture disease', 'stress myopathy' and 'polimyopathy'. Young (1972) described the clinical symptoms in tsessebe and oribi *Ourebia ourebi* as stiffness and lameness, muscular tremors, tetraplegia and torticollis. Prophylactic and subsequent symptomatic treatment with various medicaments including vitamin E and selenium-containing preparations and vitamin B_{12}, calcium borogluconate, systemic antibiotics, detoxicants, corticoids and antihistamines proved to be of no avail in clinically affected individuals.

A difficulty in the establishment of capture myopathy from muscle changes observed at autopsy, is that muscle lesions apparently identical to those resulting on overstraining may occur after death.

A condition of this nature that causes changes in muscle fibres due to denaturation which in turn interferes with the optical properties of the surface to give a characteristic pale colouring has been referred to as 'pale muscle disease' or 'pale, soft, exudative' (PSE) meat. It is stated, however, that PSE meat cannot be produced unless there is enough glycogen in the muscle for conversion to lactic acid (Anonymous 1973). Furthermore PSE develops in muscles of carcases whose pH at 45 minutes *post mortem* is less than 6,0 while the temperature is still 35 °C or higher (McLoughlin 1969), and is more prevalent in pigs stunned by captive bolt pistol than in electrically stunned pigs, the former developing a lower mean pH (Klingbiel and Naudé

1972). A decrease in muscle fibre size in carcases exhibiting low pH soon after slaughter, was attributed to poorer water-holding capacity of the myofibrillar proteins exposed to rapid *post mortem* glycolysis (Carroll 1971). The transudation of cellular fluids occurs, into the extracellular spaces of the muscle with a low pH (Dreyer, Naudé and Gouws 1972). It was pointed out by Naudé (1972) that the accelerated glycolysis in the musculature occurs *while the carcase is still warm* and that the damage does not occur before death. Although this is a *post mortem* phenomenon, it is aggravated by the degree of stress experienced by the animal directly before or during slaughter, as well as by factors occurring immediately after and leading to active muscular contraction.

To the seventeen different terms used for the various forms of muscle degeneration mentioned above, could be added Afrikaans terms commonly used such as 'ooreisingsiekte', 'vangspiersiekte', 'spier degenerasie' and 'vangspiersindroom'.

The term 'capture myopathy' has been selected as the most suitable to describe the syndrome that occurs in wild animals after capture, although it is recognised that the *post mortem* lesions may be influenced by the pre-capture nutritional state, the condition prevailing at the time of autopsy, as well as by possible intercurrent infectious disease; both the clinical symptoms and the autopsy picture tend to differ in relation to whether the condition is of the acute, subacute or more indefinite types. However, lameness and paresis appear to be constant symptoms and the term myopathy

is taken as relating to the clinical manifestations of lameness, torticollis, etc. rather than to the appearance of the muscles at autopsy.

STUDY AREAS

PHYSIOLOGICAL work was performed on live animals in several parts of the Transvaal (Fig. 1). The animals on which most of this work was performed were those captured for the purpose from various areas and kept in paddocks or enclosures in centrally situated or conveniently equipped reserves. Thus, in many instances, the geographical and climatological aspects may be irrelevant to the experiments themselves. Among the exceptions are, however, the black wildebeest at S.A. Lombard Nature Reserve, where it has now been established that a copper deficiency exists, on which mechanical capture techniques to prevent capture myopathy were investigated (Chapter Fifteen).

The physiological studies at Hans Merensky Nature Reserve were carried out on sable antelope which had been captured for eventual relocation and had already been in small pens and on artificial or supplemented food for several months prior to the experiments.

The studies using the exercise track at Percy Fyfe Nature Reserve were on animals brought from reserves as far afield as the S.A. Lombard Nature Reserve in the south western area

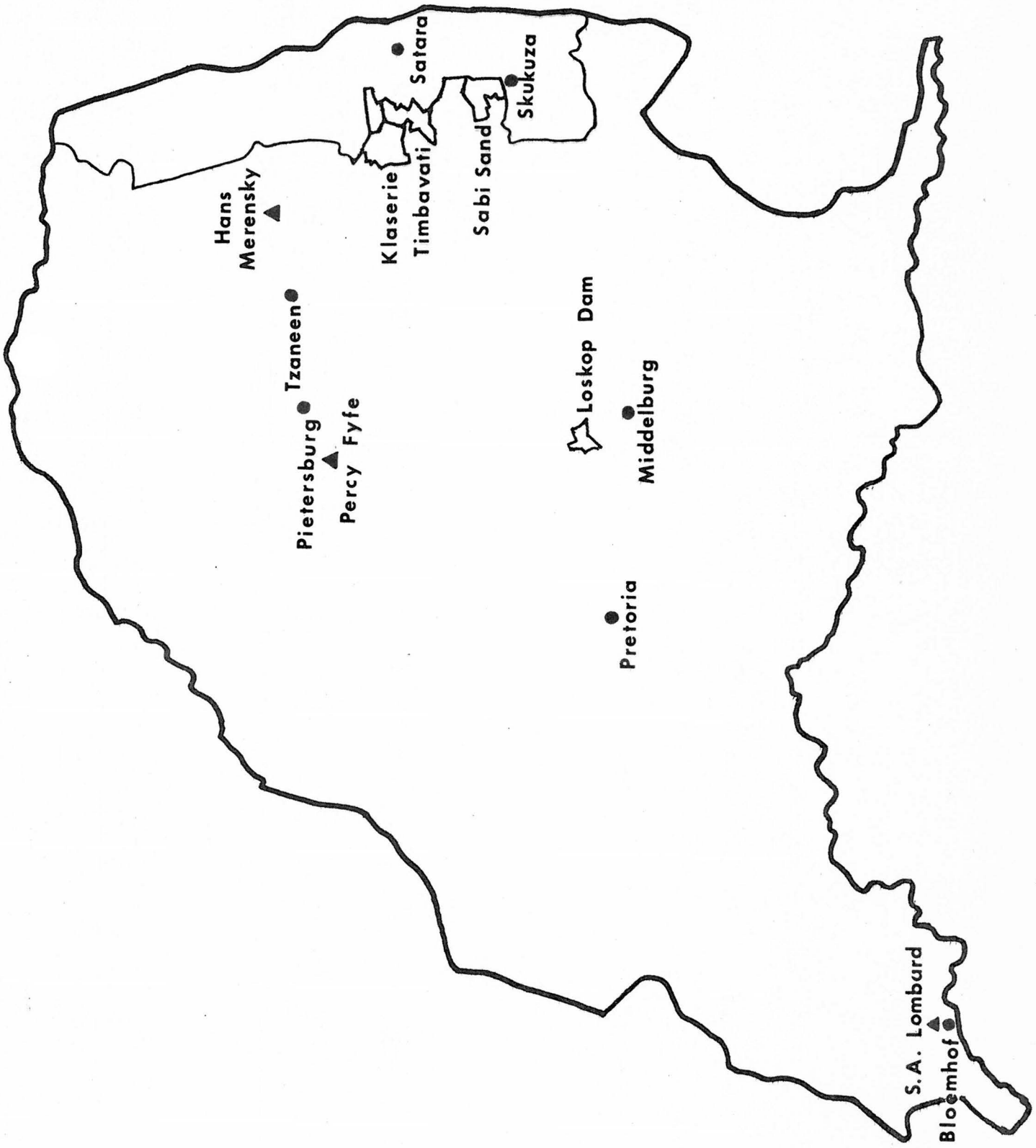


Figure 1: Map of the Transvaal showing main study areas.

of the Transvaal, and various farms in the Waterberg district in the north western Transvaal, and here again, they had been penned in small enclosures on artificial food for several months prior to the experiments.

Trace element work was carried out on liver samples taken from animals culled for various purposes unrelated to these experiments, on various areas in the Transvaal as well as the Umfolozi/Hluhluwe complex of Zululand.

The nine major areas have been tabled (Table 2) with regard to altitude, longitude and latitude as well as annual rainfall figures for the three year study period compared to norms taken over fifty years or so. The rainfall and temperature graphs are depicted in Fig. 2 to 6.

ANIMALS USED

THE animals included in this study number 104 of nine species namely, blesbok, nyala, tsessebe, blue wildebeest, black wildebeest, zebra, white rhinoceros, eland and sable antelope. Individual numbers are the following:

	females	males	total
blesbok	0	23	23
nyala	0	2	2
tsessebe	1	17	18
blue wildebeest	6	8	14
black wildebeest	0	4	4

Table 2: Geographical and climatological particulars of the main study areas.

station	lat. S	long. E	alt. M	zone†	annual rainfall (mm) 1973	1974	1975	l.t.a.††
Lunsklip	24°01′	29°07′	1475	N.T.	633,5	413,4	581,5	468,18
Satara	24°24′	31°47′	275	L.	596,3	612,3	666,4	557,6
Hluhluwe	28°05′	32°03′	452	E.	1119,7	713,8	1267,1	981,8
Mpila	28°18′	31°51′	152	E.	600,0	530,7	992,5	701,6
Bloemhof	27°39′	25°36′	1234	Sn.	469,1	718,1	510,3	482,4
Loskop Dam	25°24′	29°22′	1009	N.T.	817,5	817,5	885,7	719,7
Skukuza	24°59′	31°36′	263	L.	692,7	686,8	551,4	575,7
Eiland	23°40′	30°40′	457	L.	525,0	763,5	626,7	579,3
Jan Wassenaar Dam	24°31′	31°04′	580	L.	760,0	693,1	743,2	481,85

† N.T. = Northern Transvaal; Hot Steppe with summer rainfall (BShW mainly)
L. = Lowveld (Eastern Transvaal); Hot Steppe (Savanna) with summer rainfall (BShW)
E. = East Coast Region, Natal
Sn. = Northern Steppe (Northern Cape Province & western Orange Free State); Steppe, summer and autumn rainfall (BS(Kh)W)

†† l.t.a. = long term averages - note most figures here are above average

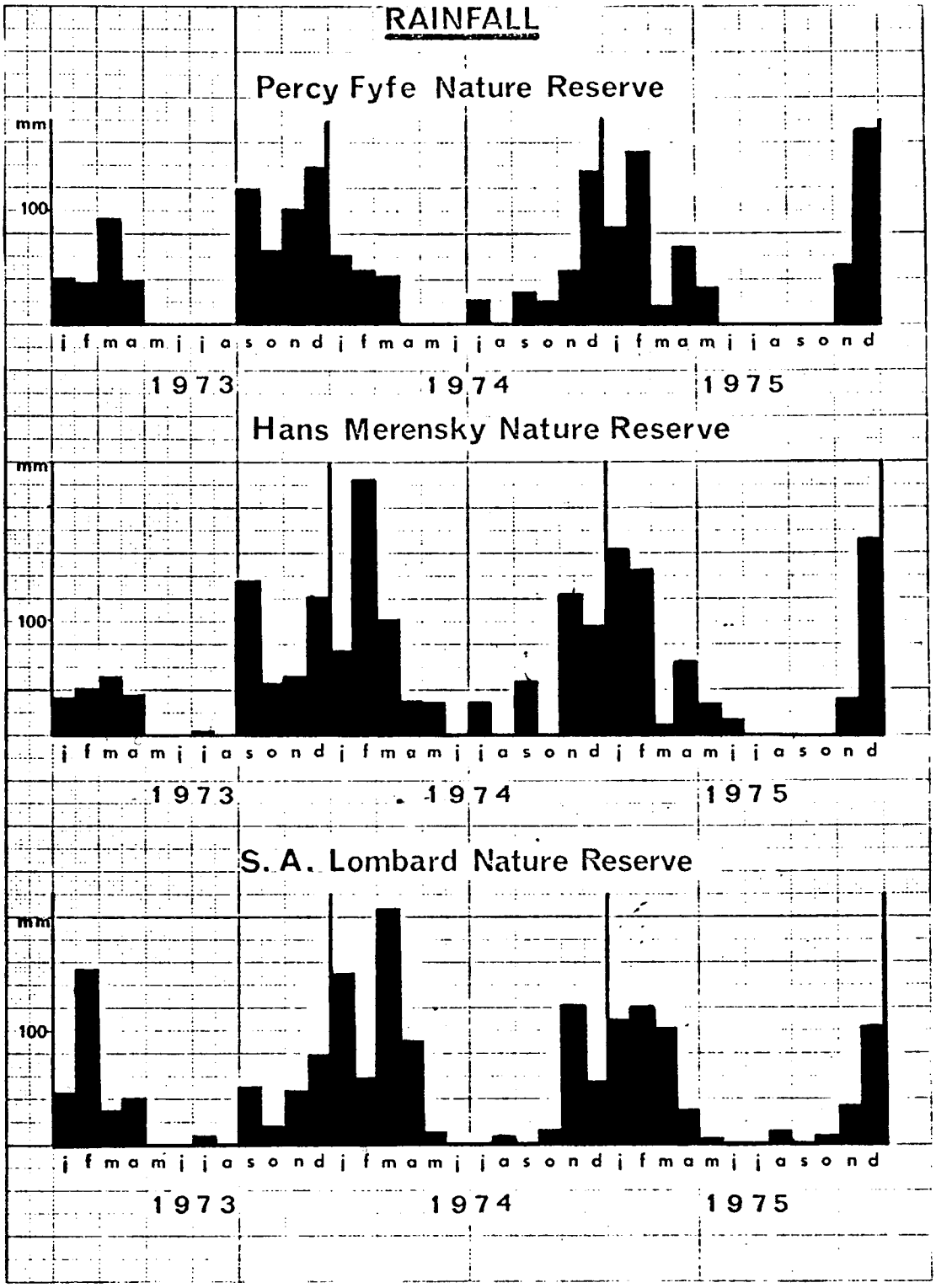


Figure 2: Rainfall in three Transvaal Provincial nature reserves.

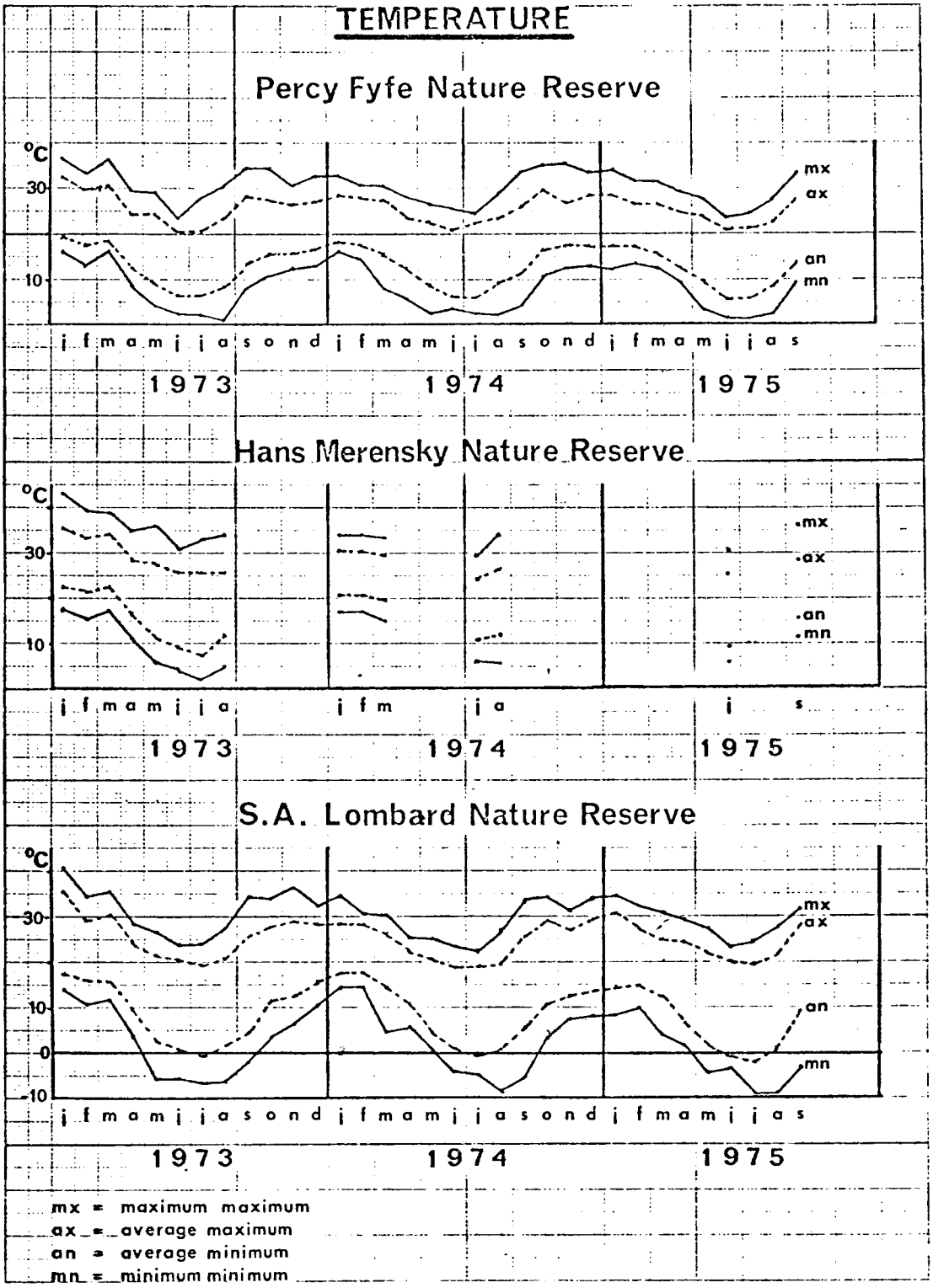


Figure 3 : Temperatures in three Transvaal Provincial nature reserves.

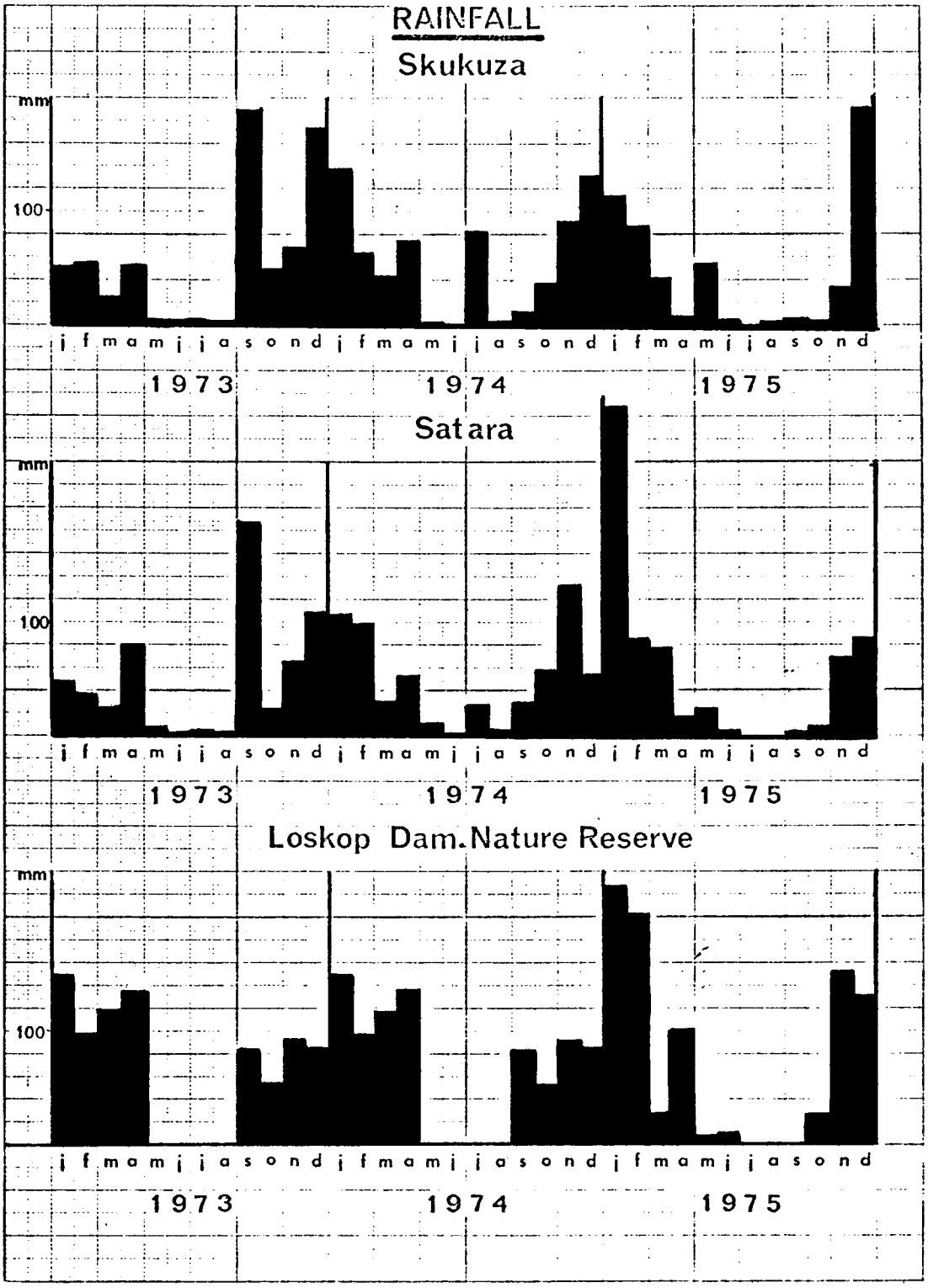


Figure 4: Rainfall in two Transvaal game and nature reserves.

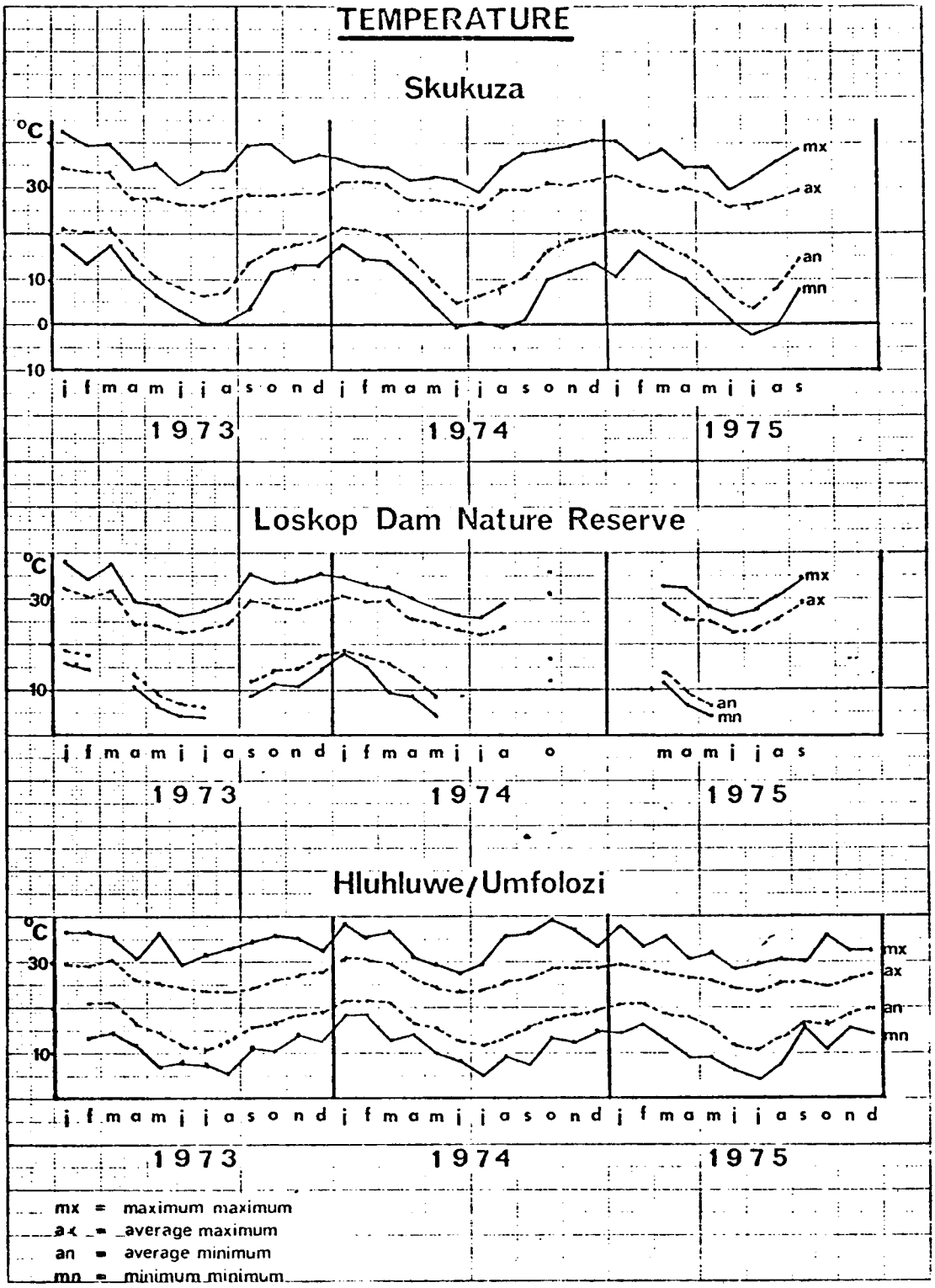


Figure 5: Temperatures in three game and nature reserves.

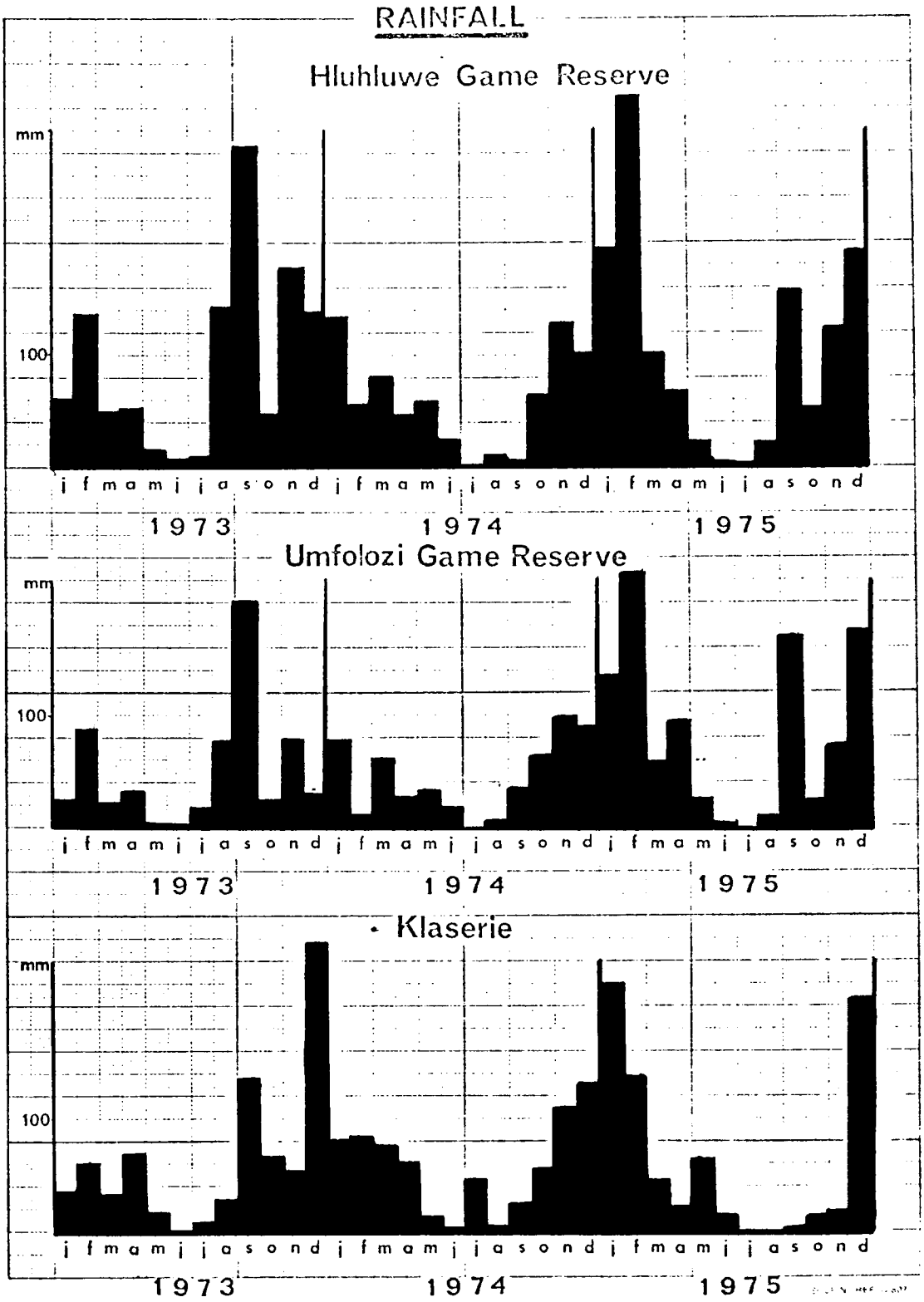


Figure 6: Rainfall in three game reserves.

	females	males	total
zebra	6	11	17
white rhinoceros	3	4	7
eland	5	5	10
sable antelope	0	9	9
	21	83	104

The number of experiments performed were 43 on a total of 4 491 samples (Table 1 for details). It should be noted that four black wildebeest are not included in Table 1 as they were used for the adaptation to stress in training experiments, and the 20 samples taken have not yet been processed.

CHAPTER TWO

TECHNIQUES

INTRODUCTION

MODIFICATIONS of existing methods were necessary, and in some cases entirely new techniques had to be evolved, to enable the work with wild animals to be carried out. Measurements of physiological parameters had to be performed on animals that tended to move or struggle. Electrocardiogram tracings were at first virtually unobtainable owing to interference until a method was developed whereby the legs and horns were wrapped in layers of plastic garbage bags so that they could be held for restraint without electrical interference from the handlers. All equipment had to be rendered completely portable so that it could be used in make-shift surrounds using current from a generator or an inverter with car battery.

INDUCTION OF LOCOMOTORY STRESS

AT an early stage into this work it was found necessary to induce locomotory stress on an experimental and controlled basis, rather than to work with animals captured for conservation purposes. The latter were often not available for

monitoring procedures and could not be examined on subsequent days and weeks.

Locomotory stress was induced in two ways. Firstly, in free-living animals mainly in the Kruger National Park by overhauling, using two vehicles over a monitored time and distance, and on one occasion by driving 18 blesbok into nets in a 600 ha paddock at Percy Fyfe Nature Reserve, and secondly, on a specially constructed track.

Free Living Wild Animals

ZEBRA and blue wildebeest were selected at random from wild herds in the Satara, Lindanda, Mananga and Mtomene areas of the Kruger National Park, and subjected to a chase of approximately 2 km distance to simulate conventional capture methods. Final capture was effected by seizing the running animal by ears and tail from both sides from the two pursuing Land Rovers or trucks. This procedure prevented any additional dyspnoea or anoxia occurring from pressure of a rope around the neck.

Immediately the animal stopped running, it was placed on its side on a plastic tarpaulin by manual restraint and the legs were tied (front and hind limbs separately, not front to back which hampers breathing), each pair of legs being restrained by two assistants, and the head held separately and blindfolded. Young animals were selected, zebras, one to two years old and yearling wildebeest, as it was found that

adult stallions and bulls could not be captured over a short distance, and when eventually caught tended to die of hyper-acute capture myopathy with blood pH values incompatible with life. As soon as the animal was restrained, measurements were made of the respiratory rates, heart rate and rectal temperature. During this procedure, the truck used as a field laboratory unit would drive to the spot. In some cases where the animals were small, and in all cases during the latter half of these experiments, the zebra or wildebeest would be put onto the truck and taken to the field unit. This latter procedure had considerable advantages in that all apparatus could be readied in the correct position for the animal's arrival; electric current switched on, and apparatus such as the electrocardiogram warmed up. The 18 blesbok were sampled for arterial and venous blood, and only clinical parameters of heart rate, respiration and body temperature were recorded.

Monitoring Procedure

The sequence of events adopted to monitoring the various parameters was the following. The order is approximate and several aspects of this procedure were usually performed simultaneously. Peripheral blood samples were taken from the recurrent tarsal vein in 'Venoject' tubes. The jugular furrow was clipped, cleansed and disinfected, as well as the part of the right flank for aortic puncture. The jugular vein was then punctured with a 12 gauge needle and a 'PE 200 Intramedic' catheter passed via the jugular vein into the right heart. Local anaesthetic was induced

in the area for aortic puncture. A small stab wound was made to insert the needle for aortic puncture, through which a 'PE 50' or a 'PE 100 Intramedic' catheter was passed into the posterior aorta. Mixed venous, arterial and capillary (from ear capillaries) blood samples were taken at regular intervals. The electrocardiogram leads were connected to the chest and limbs. Electrocardiogram, venous, atrial, ventricular and pulmonary artery pressures were monitored. Systemic blood pressure was measured simultaneously with the electrocardiogram. Environmental temperature and relative humidity were taken. A second catheter was inserted into the jugular vein and passed into the pulmonary artery, after which cardiac output was computed. Rectal temperatures were compared with core temperatures taken in the pulmonary artery.

These procedures were repeated as far as possible every 30 minutes. After the animal was treated and observations carried out for a further hour to hour-and-a-half, the animals were either clearly marked and released, or numbered and taken back to the pens at Skukuza for continued observation and regular sampling.

A similar procedure was carried out during the capture of the 18 blesbok in paddocks in the nature reserve.

Captive Animals

Animals were released from pens or driven from small holding

paddocks singly into the small circle of the figure of eight exercise track. The vehicle used for chasing had previously been stationed in the large circle (see section on the exercise track in the Chapter). A gate was then opened long enough to allow the animal to move from the small to the large circle just in front of the vehicle which then moved around the track at a slow speed. As soon as the animal passed a monitoring point, the time was recorded with a stopwatch and the vehicle accelerated to 30 km/h. Sixteen laps were then made at a speed as near as possible to the 30 km/h although the animals usually slowed down considerably towards the end. At the termination of sixteen laps of 125 m each, a gate was opened to channel the animal into the small circle. There it was permitted to slow down and eventually be caught in a wooden crush. It was blindfolded while standing up and led into the area inside the small circle where straw bedding covered by a tarpaulin and shaded by a second tarpaulin had been prepared. Tables were available for glassware, although the instruments were run from the back of the laboratory unit as for free-living animals. An electric fan for cooling the animals was used during the hot weather so that work during the summer was possible.

Casting was performed manually in the case of small antelope and Reuff's method (Fig. 7) of casting was used for eland. The procedure then followed that for free-living animals, with variations according to the object of the particular experiment.

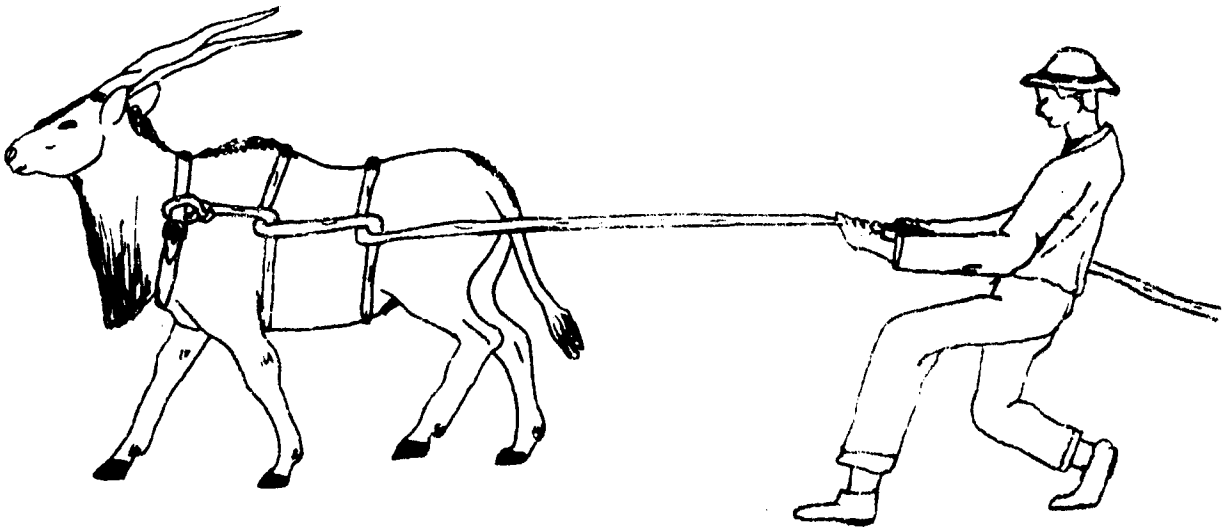


Figure 7: Reuff's method of casting applied to tamed eland at Percy Fyfe Nature Reserve.

Sampling (twice weekly at first, with larger intervals as the parameters approached normal) for peripheral blood was performed in the standing position using the manual restraint and a blindfold on animals such as tsessebe, and after casting for animals such as eland. Neither procedure appeared to influence the smooth transition of enzymes to normal levels.

THE EXERCISE TRACK

DIMENSIONS OF TRACK

The general shape of the structure is that of a figure of eight. The top half of the figure consists of the main exercise track which surrounds a large paddock. The lower half of the 'eight' is formed by a run-off track which is smaller in width and length and also encloses a paddock. There are a number of gates strategically placed to facilitate direction of run as desired (Fig. 8 to 11).

The structure covers a total area of 2 116,9 m^2. The area of the main paddock with track is 1 362,5 m^2, the diameter of the paddock being 39 m and the track itself being 290 cm wide. The run-off track is one metre wide enclosing a paddock of 30 m diameter. The crush is 70 cm wide.

The length of the main track is 125 m, and therefore eight laps are needed to complete a 1 km run. The run-off track is 95 m long and leads into the corridor adjacent to the holding pens.

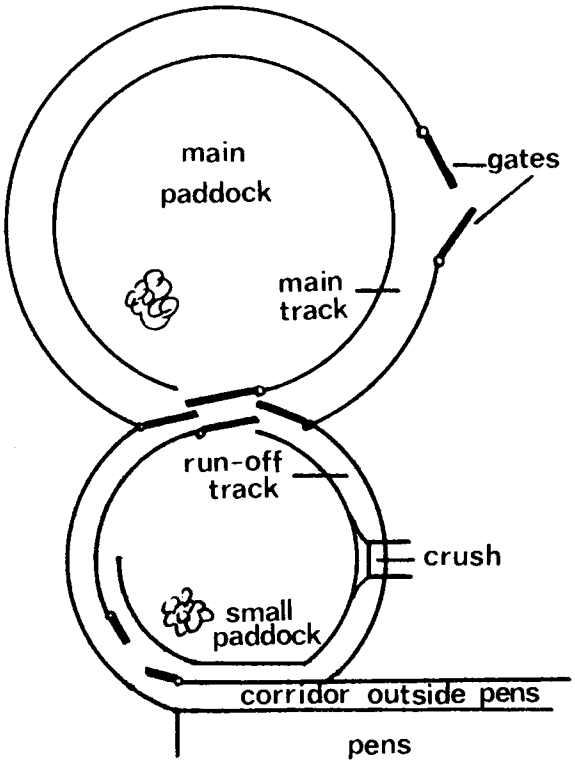


Figure 8: Plan of exercise track for the production of controlled locomotory exercise situated at Percy Fyfe Nature Reserve.

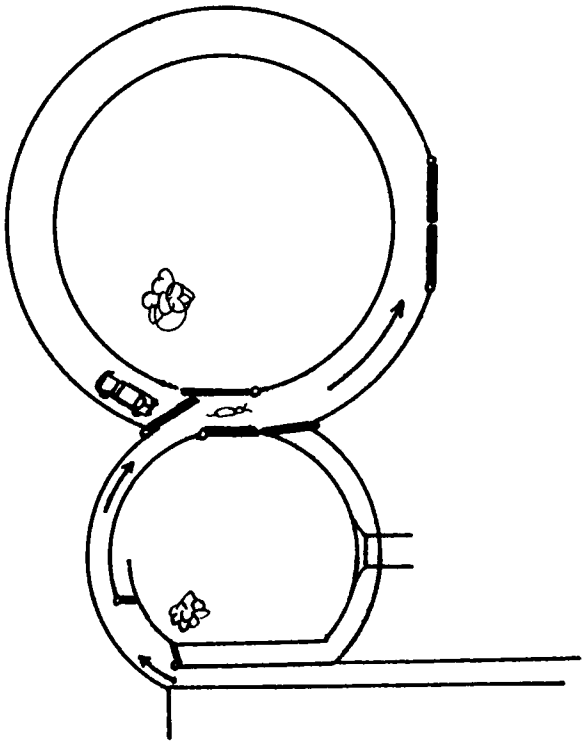


Figure 9: Position of gates of exercise track prior to chase.

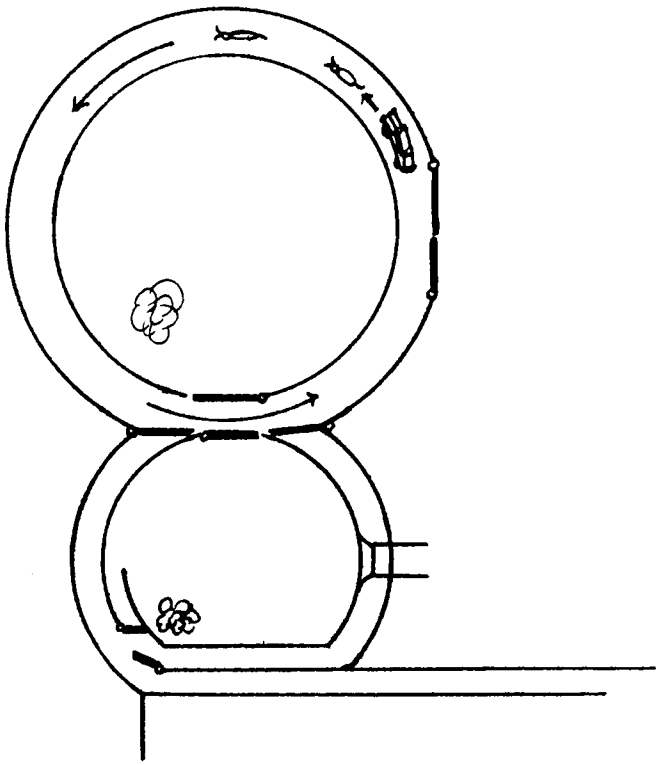


Figure 10: Position of gates of exercise track during chase.

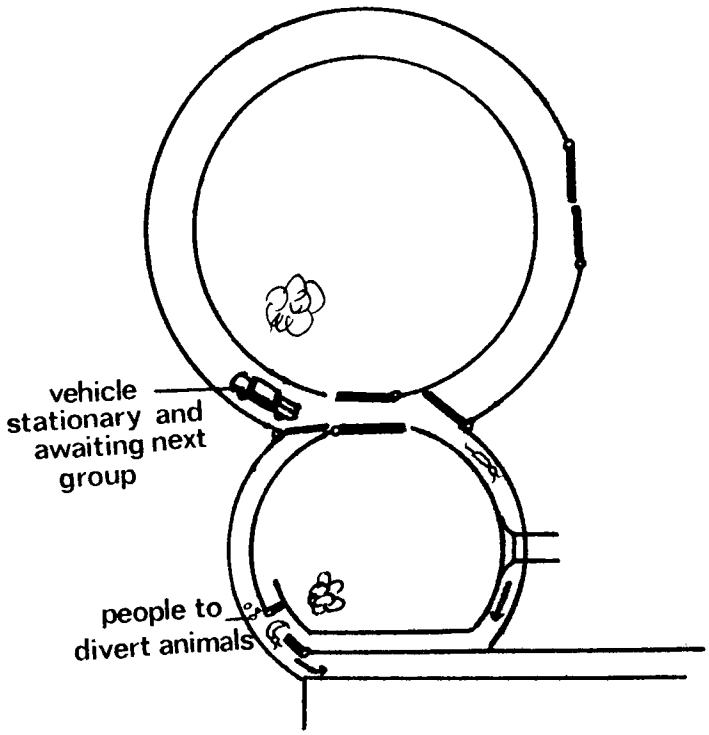


Figure 11: Position of gates of exercise track after chase.

The height of the walls and the gates, is 245 cm. The circumference of the main paddock is 122,5 m with the smaller paddock having a circumference of 94,2 m.

As river reeds were used to cover both the walls and the gates, it is difficult to discern without close inspection, from inside the track itself, where the wall ends and the gates begin. This is advantageous as it prevents the animals being distracted, thus facilitating a smooth and controlled chase. Inside the paddocks are several trees for shade, and food and drinking troughs when used for holding animals.

MATERIALS

There are six large gates 245 × 308 cm (8' high and 10' wide) and two gates measuring 245 × 100 cm (8' high and 3' 3" wide). One hundred and fifty solignum poles were used, measuring 308 cm (10'), 245 cm (8') of which is above the ground. These are 13 cm (5,5 inches) in diameter, except for the poles used as supports by the gates, which are 16 cm (6,5 inches) in diameter. Along the walls they are placed two metres apart.

Twenty tons (metric) of locally acquired river reeds were used to cover the walls and gates. Other materials are items such as high tensile fencing wire, barbed wire, mesh wire, etc.

Adrenaline Stress In Immobilised Sable

A series of nine captive surplus sable antelope bulls were immobilised and subjected to a slow infusion of adrenaline in physiological saline solution (Chapter Eleven).

One animal only was handled on any day. The door of the pen was opened to give access to a corridor ending in a crush. Once in the crush the animal was injected with a mixture of fentanyl citrate, azaperone and xylazine hydrochloride at the dosage rates tabled in Appendix A.

As soon as the drugs took effect the animal was taken from a ramp onto a pick-up truck and transported 200 m to a suitable area equipped with a table, electric power socket and shade. Here the animal was placed in right lateral recumbency with the head and neck on a grass-filled bag and with the mouth lower than the rest of the head. Light immobilisation was used and the animal restrained in a manner similar to that described above except that each leg was held separately and particular care was taken to insulate the animal from the handlers so as to obtain trustworthy electrocardiogram tracings.

The jugular furrow was then clipped, cleansed and disinfected and similarly the skin of the recurrent tarsal vein. A catheter was then inserted into the latter for regular sampling of peripheral blood and for infusion. Catheters were inserted into the jugular vein for blood pressure recordings and cardiac output estimations. Systemic pressure

was determined using a sphygmomanometer cuff and stethoscope. Rectal temperatures were recorded with a clinical thermometer, and checked against the core temperature from the pulmonary artery. All parameters were monitored every half hour for several hours. In all cases it was necessary to use an electric fan and to moisten the body on account of a rapid and sustained rise in body temperature.

INFUSION

THE infusion administered to free-living animals approximately one hour after capture consisted of one litre physiological saline or 'Normosol' (a balanced solution of blood crystalloids - Chapter Four) to which 1 000 m-equiv. sodium bicarbonate had been added. The liquid was infused into a superficial leg vein in two equal parts. Blood samples for pH (as well as PO_2, PCO_2, haematocrit, plasma bicarbonate, etc.) were taken just before infusion, five minutes after infusion of 500 m-equiv. and five minutes after completion of infusion. Capillary samples were taken as well as the central mixed venous and arterial samples for haematocrit, PO_2 and PCO_2 estimations, before, during and after infusion.

Adrenaline was administered as 2 mg adrenaline per litre saline given over 60 minutes, followed in most cases by another, or part of another infusion prepared in the same way as infused into the recurrent tarsal vein through an

indwelling catheter.

All infusions were prepared in one-litre 'Flex-flac' disposable soft infusion packs. The bicarbonate was added previously in the laboratory, the pack resealed and clearly marked. The adrenaline was added one hour or so before infusion.

CLINICAL PARAMETERS

HEART rates were determined by direct auscultation using a stethoscope and stop-watch over half a minute. An attempt was made at the same time to determine whether abnormal heart sounds or cardiac dilatation was in evidence. The character of the heart sounds was noted as well as the rate on sheets prepared for the purpose of these experiments (Appendix B).

Respirations were counted by direct observation of the flank over one minute using the second-hand of a wrist watch. The character of the respirations was also noted and any degree of cyanosis by observation of the conjunctiva.

Body temperature was recorded using a clinical thermometer pushed completely into the rectum. These observations, made every half-hour were checked at regular intervals with the core temperature. The latter was taken with a thermistor in the pulmonary artery which was observed from a meter

on the cardiac output computer. The thermistor was mounted on the extreme tip of a catheter and the location in the pulmonary artery was checked by the character of the recorded pulse waves.

In addition to the parameters mentioned above, which were recorded every half-hour, regular checks were made for signs of bloat, dyspnoea or cyanosis, possible obstruction of breathing and so forth.

PHYSIOLOGICAL PARAMETERS

BLOOD PRESSURES AND INSERTION OF CATHETERS

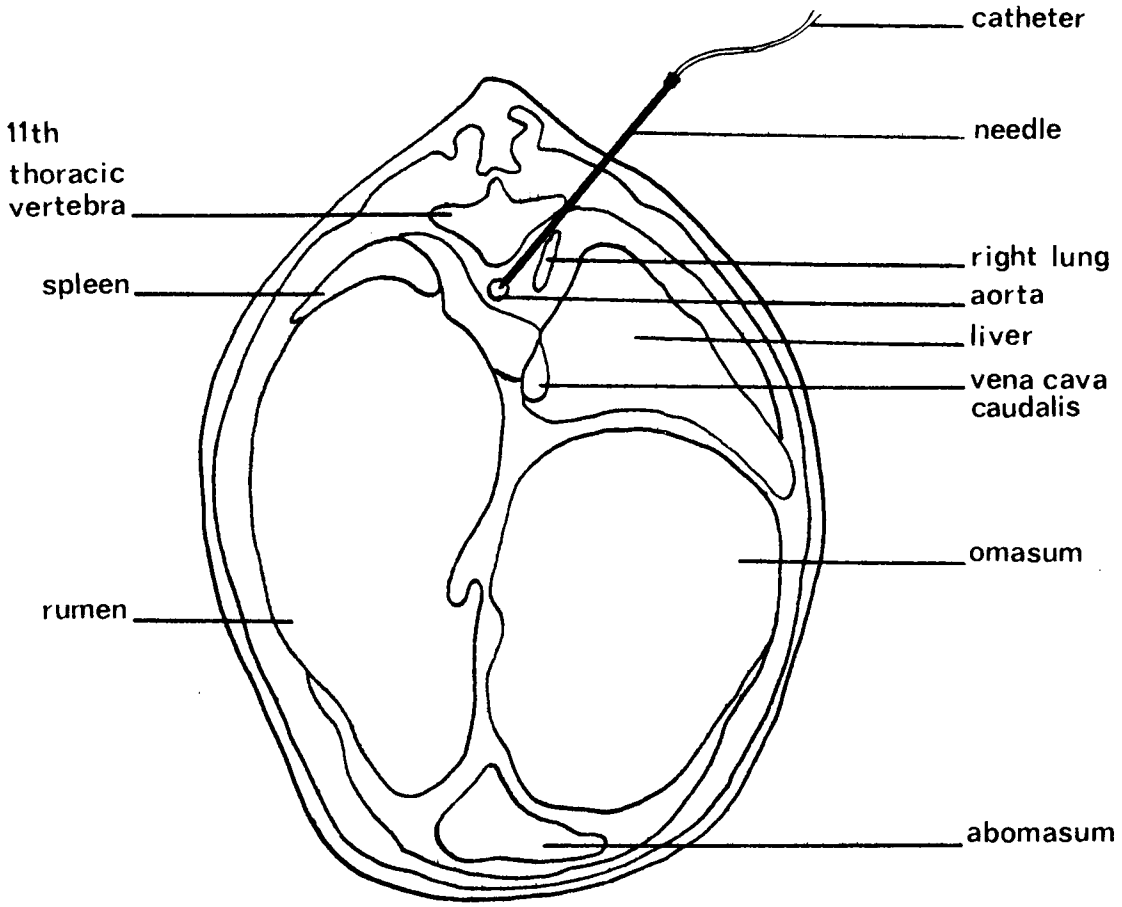
VENOUS, RIGHT ATRIAL AND VENTRICULAR, AND PULMONARY ARTERY BLOOD PRESSURES

A 'PE 200 Intramedic' catheter was passed through a 12 gauge needle inserted into the jugular vein. The proximal end of the catheter was equipped with a 'Clay-Adams' plastic catheter adaptor for luer syringes or for a pressure transducer. The distal end of the catheter was notched to prevent possible sealing by a small blood clot acting as a valve, and small pin-holes through the wall, five centimetres from the end to reduce rebound. The catheter was carefully filled with heparinised saline before insertion, and distances marked along its length using an 'Artline' waterproof felt-tipped pen.

Entry into the ventricle was usually obvious from the thrill of pulsations transmitted to the free end of the catheter. Central venous blood samples were taken before flushing and connecting to a 'Bell and Howell' transducer and 'Devices' recording module. The transducer was laid on a sand-bag placed behind the animal at heart level. Subsequent blood samples and flushing and repeated determination of the base line was carried out through the three-way tap system situated between the 'Clay-Adams' adaptor and the transducer. The location of the catheter in the vein, heart or pulmonary artery was readily indicated by the characteristic pulse-waves on the recording paper.

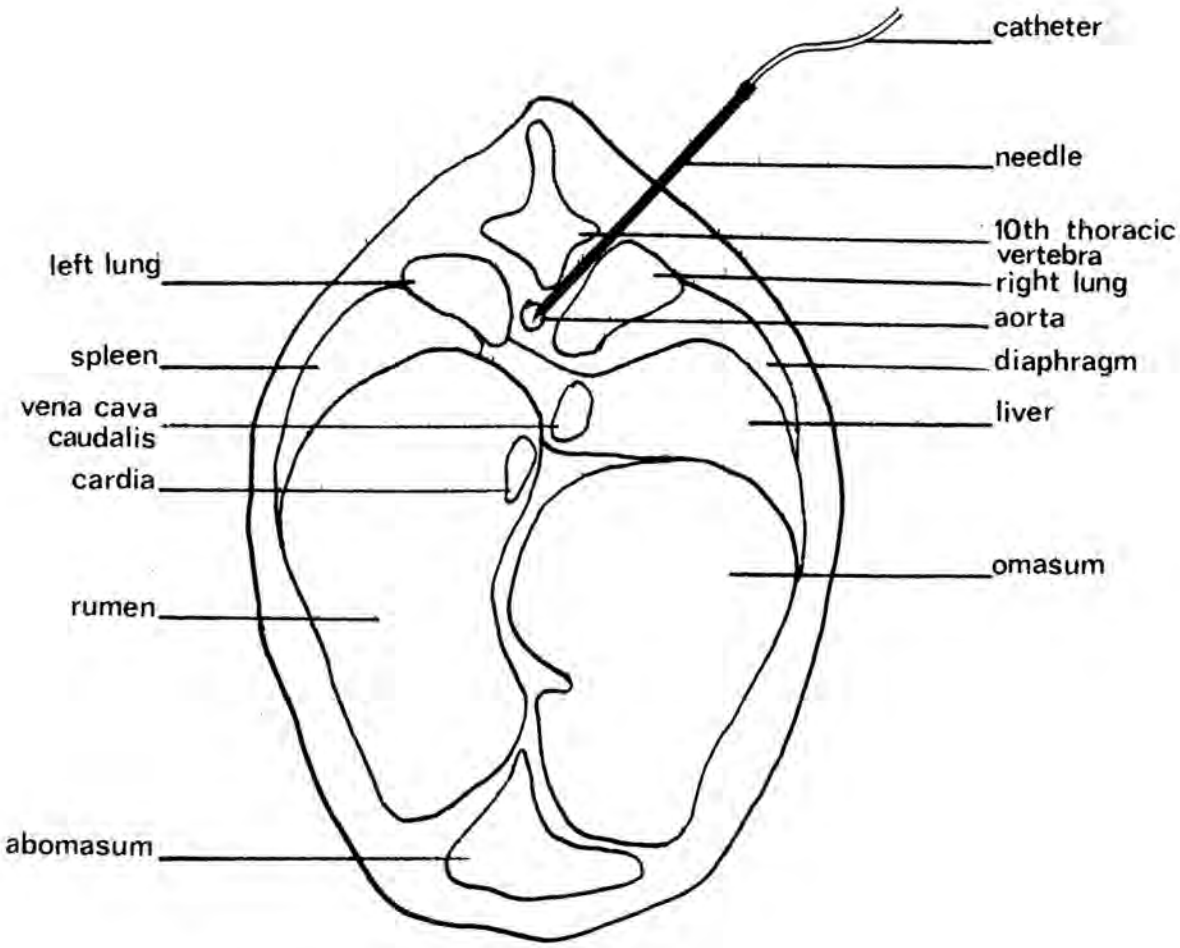
Systemic Blood Pressure

The skin area to be punctured was clipped, cleansed with 'Savlon', detergent soap and disinfected with 70 percent ethyl alcohol and anaesthetised with procaine hydrochloride before making a small stab wound with a 'Bard-Parker rib-back no. 10' pointed scalpel blade. The puncture wound prevents deformation of the needle on entry, and facilitates easy movement and therefore sensitive exploration of the tissues to feel the throb of the posterior aorta. For early work, a 'PE 100 Intramedic' catheter was passed through a 28 cm long 15 gauge needle (custom-built) inserted into the posterior aorta through the upper right thirteenth intercostal space in zebra and the ninth in ruminants (Fig. 12 to 15). After a death due to sudden uncontrolled struggling in one zebra, a 'PE 50' Intramedic' catheter was used through a 17 gauge needle. In each case, the needle was withdrawn as rapidly



Puncture of the aorta in this region avoids damage to vital organs such as the kidney which lies caudad. After puncture the needle is replaced by a catheter pointing cephalically towards the heart.
(adapted from Lagerlöf 1930)

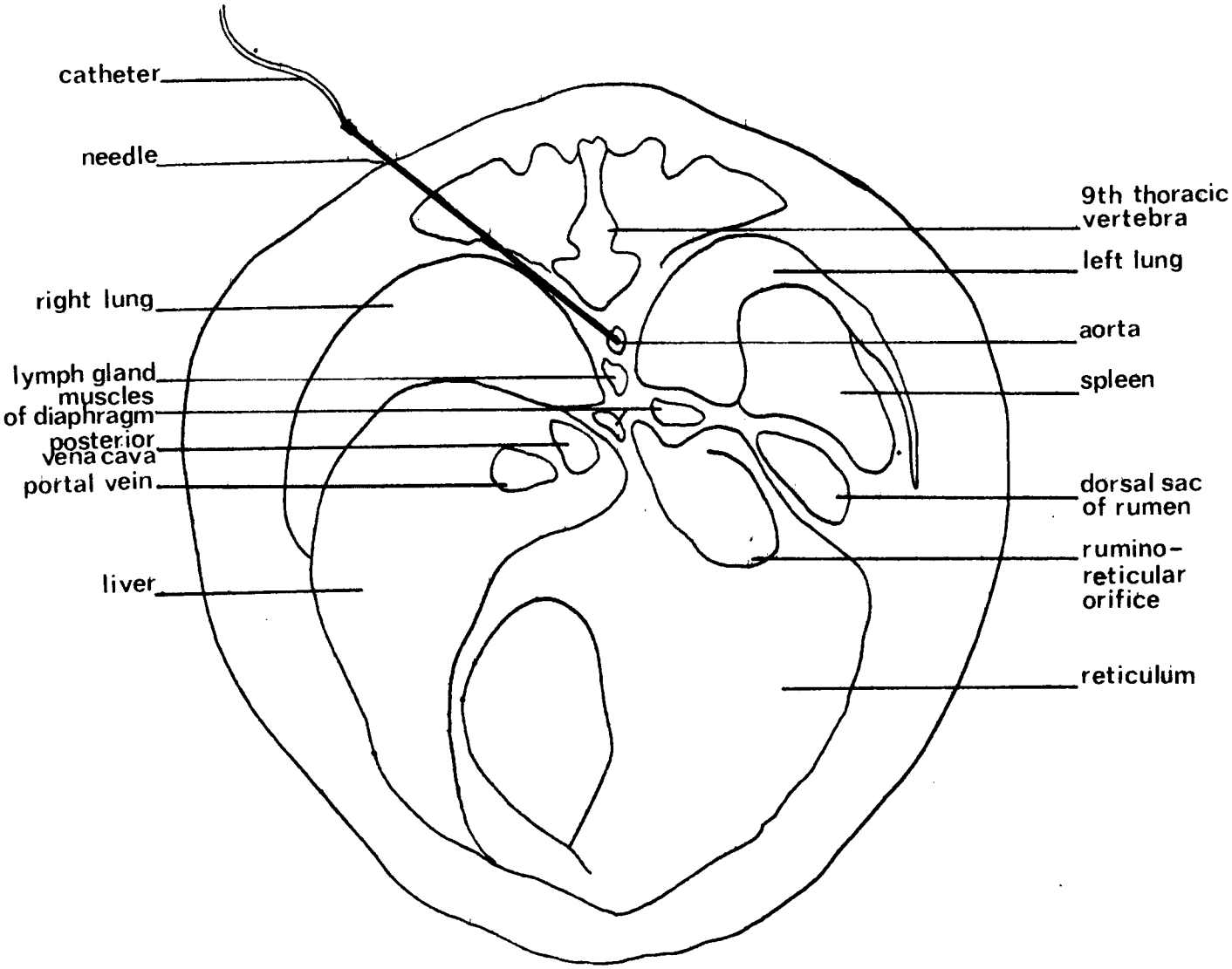
Figure 12: Section through 11th thoracic vertebra in ruminant showing position of needle and catheter.



A position slightly more cephalic facilitates aortic puncture. The needle is likely to traverse part of the lung but without ill effect. An attempt is made to remain retroperitoneal.
(adapted from Lagerlöf 1930)

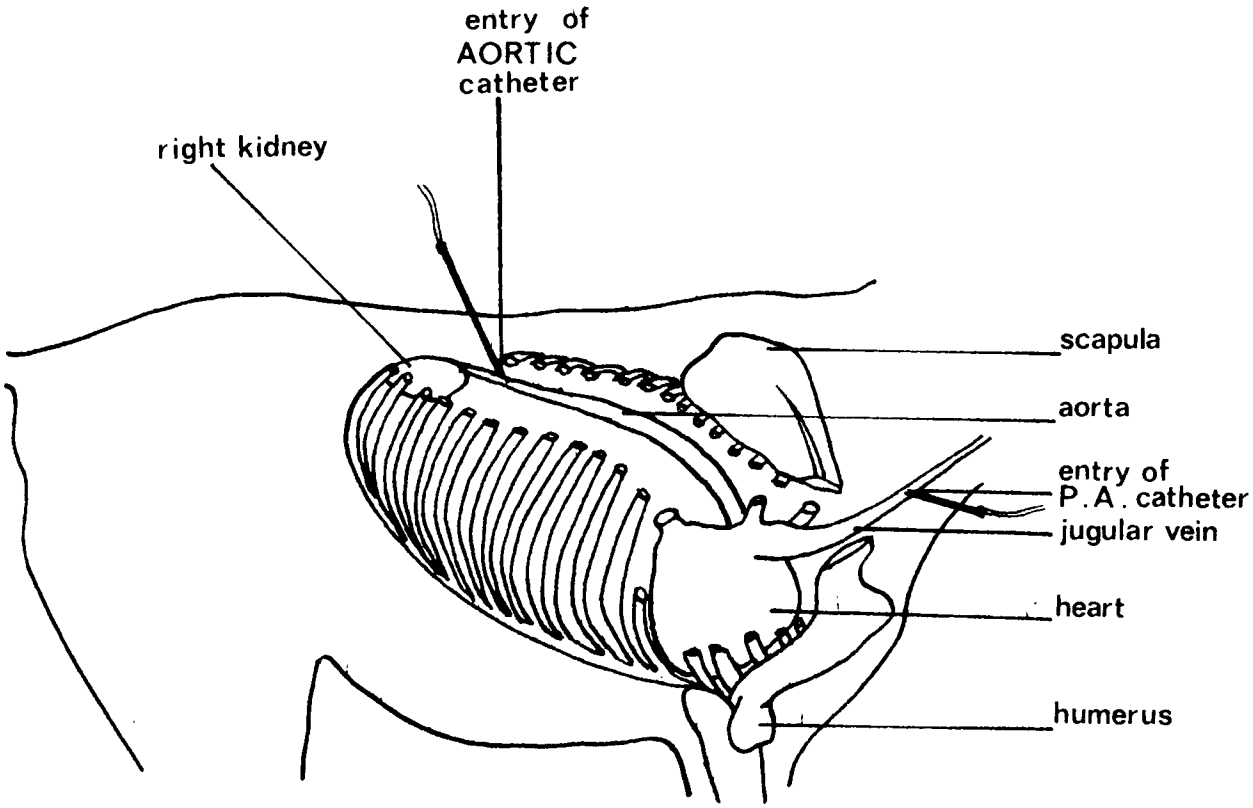
Figure 13: Section through 10th thoracic vertebra in ruminant showing position of needle and catheter.

ANTERIOR VIEW



Puncture at this level (between the 9th and 10th ribs) entirely avoids the liver but is likely to involve the lung.
(adapted from Lagerlöf 1930)

Figure 14: Section through 9th thoracic vertebra in ruminant.



This diagram illustrates how both the right and the left sides of the heart may be catheterised. The catheter on the right side passes through the heart into the pulmonary artery, but the tip of the aortic catheter is placed in the aortic arch.
(adapted from Sisson and Grossman 1947)

Figure 15: Positions of pulmonary artery and aortic catheters in zebra.

as possible after puncture. The saline-filled catheter was inserted into the needle before puncture of the aorta to prevent massive back-bleeding and on puncture of the vessel passed for approximately 50 cm cranially before withdrawing the needle. The needle and catheter were then taped onto the skin alongside the spine, so as to immobilise the catheter and eliminate drag from the weight of the needle. The catheter was connected to the recording module in the way described for pulmonary artery pressure recording. In addition this catheter and the three-way tap assembly (Fig. 16) subserves the dual purpose of recording the blood pressure on a semi-continuous basis on the same paper strip as the electrocardiogram, and for carefully timed collection of arterial blood samples. Where this procedure was not followed as in small antelope, arterial samples were obtained by direct puncture of a superficial artery using a 2 cc glass syringe and a 22 gauge 50 mm needle.

ELECTROCARDIOGRAM

THE electrocardiogram was recorded with a 'Devices' recorder on heat-sensitive paper at speeds of 10 to 25 mm per second. For skin electrodes, 15 gauge 60 mm needles were used inserted under the skin near and just above the carpus and tarsus and near the apex area of the heart on the chest and held in place by two silk sutures; one picking up the needle near the point and under the skin, and the other over the boss. All leads (ten) were run, but for regular

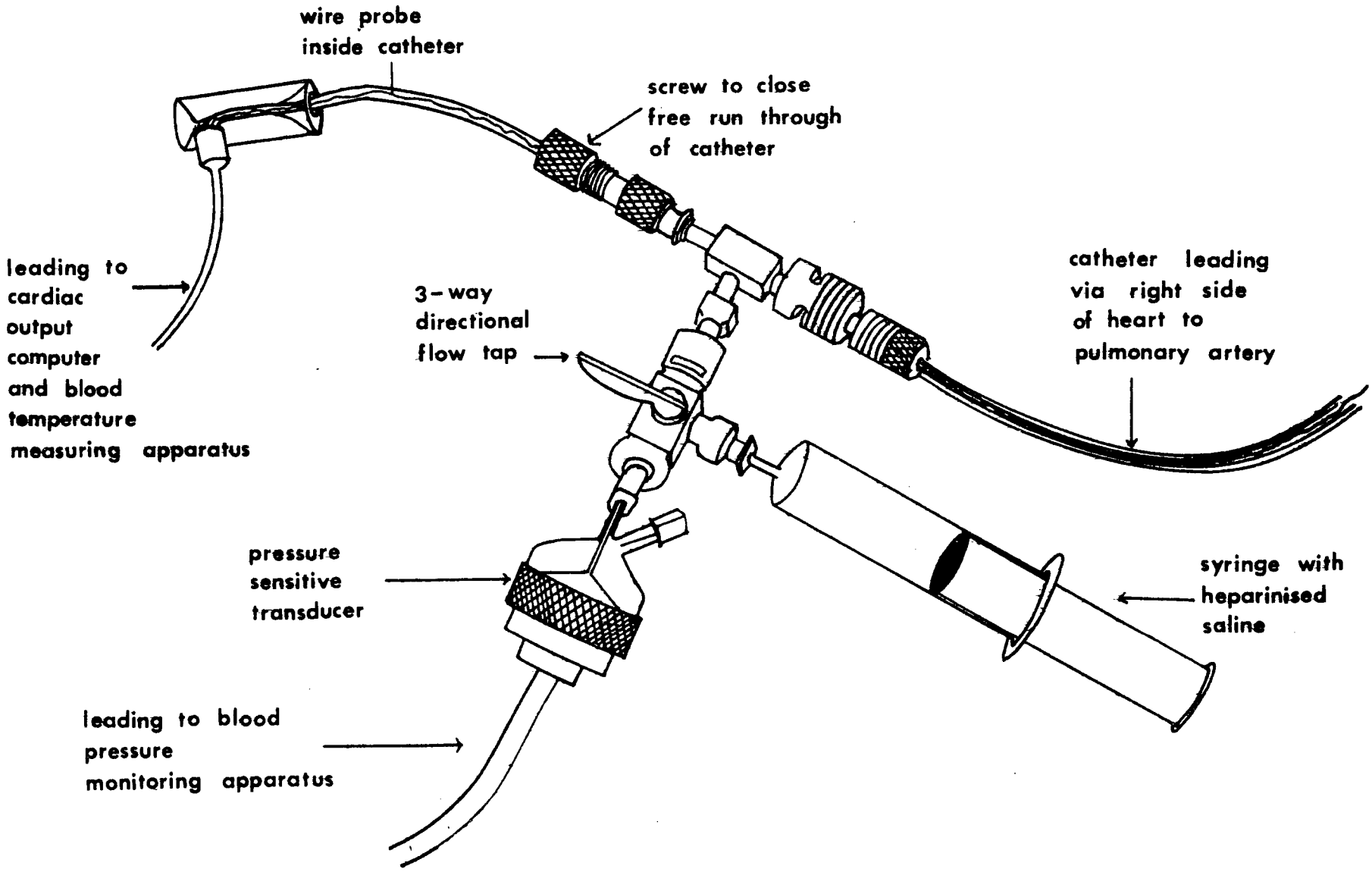


Figure 16: Three-way directional tap system for the simultaneous measurement of blood pressure and cardiac output.

half-hourly recordings, one lead only was used, usually lead number two.

CARDIAC OUTPUT

CARDIAC output was measured using a 'St Thomas Hospital' cardiac output computer ('Devices') working on the principle of thermal dilution. Most of the work was performed with a borrowed instrument which necessitated two catheters in the pulmonary artery. The second catheter (B) was therefore passed into the jugular vein well caudal to the first to enable a short probe to reach the correct distance. This latter catheter contained a heat-sensitive thermistor probe. When this catheter was in the correct position as indicated by the conformation of the pressure waves, the original catheter (A) was partly withdrawn so that its tip - according to the distance markings made on the catheter (see above) - was lying at a distance of 15 cm behind the tip of catheter B containing the probe.

The reference electrode of the computer was placed in saline at a temperature of 18 to 20 °C placed in a 500 cc beaker inside a 'Thermos' vacuum jar. The cardiac output was measured by injecting 10 ml of this saline (with a syringe previously placed in the saline solution to acquire a same temperature) into catheter A whereupon the thermal dilution was measured by the probe in catheter B.

The catheter provided with the instrument and carrying the

heat-sensitive probe proved unsuitable for use with the test animals, being too thin to carry adequately the wave formations necessary to determine the location in the pulmonary artery, and other problems. The thin catheter and probe were therefore placed inside a 'PE 200 Intramedic' catheter as routinely used for registering pulmonary artery pressures. In this manner the probe was readily introduced into the pulmonary artery, and the positions of the tips of each of the two 'PE 200 Intramedic' catheters readily assessed.

To enable a recording to be made through this assembly a special adaptation was constructed from a combination of T-pieces and three way tap as depicted in Fig. 16. This adaptation enabled the pulmonary artery pressure to be recorded through the 'PE 200 Intramedic' catheter without loss of pressure in the catheter which causes back-bleeding along the probe, and subsequent clotting as the heparinised saline is lost. This modification worked well and proved superior for our purposes to the multiple catheter assembly supplied with a later cardiac output computer subsequently used.

Core Temperature

Core temperature was recorded in the pulmonary artery using the thermistor probe of the cardiac output computer. As previously established (Bligh and Harthoorn 1965) the rectal and core temperature fluctuate in respect of each other but at no recorded point differed by more than 1 °C, and usually

showed much closer correlation. These results were used to corroborate the efficiency of rectal temperature measurements in the manner used, i.e. the clinical thermometer was placed completely inside the rectum and left in that position for a time not less than one minute.

BLOOD SAMPLING

BLOOD samples were taken in four different ways:-

CENTRAL VENOUS BLOOD SAMPLES FOR pH, PO_2, PCO_2 AND HAEMATOCRIT DETERMINATIONS, AND ARTERIAL SAMPLES DRAWN FROM THE POSTERIOR AORTA

FOR these purposes 5 cc polypropylene disposable syringes were used. The dead-space of the syringe was filled with heparin solution BP 5 000 I.U./ml. Exactly 5 ml blood was drawn to ensure standard dilution of the heparin; a factor of less than one percent. The syringe was immediately closed with a small rubber bung on the end of the needle. The syringe had been previously labelled,and then enclosed in a small plastic bag before being placed in ice-water carried in 4,5 litre vacuum jars.

Immediately on taking the sample the syringe was rolled between the palms of the hands or twirled by the boss of the needle to ensure thorough mixing of the blood with the heparin solution.

Peripheral Arterial Samples

Where the aorta was not catheterised, arterial samples were obtained by puncture of a superficial artery on the medial surface of a limb. For this purpose it was necessary to use a syringe where the plunger moved with a minimum of friction so that the force of the arterial blood was able to fill the syringe immediately the vessel was punctured. For this purpose 2 cc glass syringes were used with 22 gauge 50 mm needles. Free movement of the plunger was ascertained after moistening with heparin before use. The dead-space was filled with heparin solution and procedure followed as for central blood samples above.

Peripheral Venous Samples

Peripheral blood for blood enzymes and for metabolite estimation was drawn from a superficial vein, usually the recurrent tarsal, without stasis and using a 'Venoject' 10 ml vacuum tube assembly. Heparinised tubes were used for lactate, and plain tubes to provide serum for CPK, LDH, GOT and GPT estimation, serum being more stable than plasma (see below), as well as blood urea nitrogen, urea and creatinine. For certain experiments only, namely for the infusion work on sable antelope where a large number of peripheral samples were required exceeding that of other experiments, and to facilitate infusion, peripheral blood was drawn into 5 cc syringes from the recurrent tarsal vein using an indwelling 'Steril-Peel Desaret Intercath' intravenous

catheter so to preclude excessive number of venepunctures and destruction of the vein.

Capillary Samples And Microhaematocrits

Capillary blood was taken from the ear after depilation and careful shaving off the outer skin layers of a small area on the edge of the pinna. 'Heraeus Christ' heparinised capillary tubes were used for haematocrit determination, using both capillary blood and central venous blood collected as above. Capillary blood for 'Astrup' work was collected using 'Radiometer' heparinised capillary tubes. A mixing wire 1 cm long was inserted and the ends of the tubes sealed with plasticine. A magnet was passed rapidly up and down the length of the tube to activate the mixing wire and ensure thorough mixing of the blood and heparin. The tubes were labelled in batches and placed in ice-water as for syringes. The small series of samples for capillary blood vessel microhaematocrit determinations using siliconised hard glass capillary tubes were heat sealed at the upper (dry) end, and stored vertically Routine samples for haematocrit determination were taken from the central blood after collection in a syringe. Prior to filling the haematocrit tube the syringe was again thoroughly agitated by rotation to reverse the effects of sedimentation, and the first few drops from the nozzle discarded before introduction of the end of the capillary tube. Haematocrit values were determined in a microfuge holding 24 micro-haematocrit tubes and spinning at 14 000 revolutions per minute for ten

minutes. The values were then read with a microhaematocrit reader.

TESTS FOR STABILITY IN SERUM AND PLASMA

Introduction

DELAYS were often experienced in getting samples back to the base laboratory for analysis. In spite of carrying all plasma and serum samples in vacuum jars on ice at all times and storing these at sub-zero temperatures, degenerative changes in the serum and changes in the levels of various blood constituents became evident. It was therefore decided to establish the rate at which these changes took place. As it is customary to use either plasma or serum for tests on the relevant blood constituents such as LDH, GOT, etc., changes in both plasma and serum have been tested.

Materials And Methods

ALL blood samples were placed in ice-water immediately after collection and centrifuged within one hour. Only samples free from haemolysis were used. Three enzymes were considered separately. The differences in values were compared from the first day of analysis to the 11th, 12th or 13th day of analysis, in both plasma and serum.

RESULTS

THE statistical results are as follows:

GOT : plasma t_4 = 2,426** serum t_4 = 1,278 not significant

GPT : plasma t_4 = 2,818** serum t_4 = 0,997 not significant

LDH : plasma t_4 = 0,596 serum t_4 = 1,117 not significant
not significant

The signed rank test of Wilcoxon lead to the same conclusions. The observations can be considered as normally distributed within reasonable limits according to the rankits graph plotted for these values.

DISCUSSION

FOR GOT and GPT, serum appears to be significantly more stable than plasma over a period of time for the purpose of analysis. There is no significant difference in the LDH results.

MEASUREMENT OF PO_2, PCO_2 AND pH

THESE parameters were measured with the use of an 'Astrup' radiometer and pH meter. The equipment comprised a radiometer with built-in waterbath, suction pump, saline and other reservoirs, rinser, also pH (capillary), PO_2 and PCO_2 electrodes, humidifiers and gas selector. The radiometer was coupled to a pH meter with a visual digital display for pH, PCO_2 and PO_2. Attached to the radiometer was a

** denote levels of significance - see 'Statistical Methods' in this chapter.

manometer (gas mixing apparatus) for mixing required concentrations of air and CO_2 pressures. The manometer was linked up to medical cylinders of air and CO_2.

The radiometer and pH meter were set up as near as possible to the experimental site wherever mains electric power was available. The blood samples were analysed at a temperature of 38 °C and corrections were made for changes in acidity according to the time the blood remained in ice-water between drawing and analysis. The measurement of the blood samples involved three determinations. Firstly, the pH was measured on the anaerobic blood sample after this had been drawn into the capillary electrode which requires a minimum of 25 µl blood. The measurements were made against two precision buffers at a pH of 6,841 and 7,383 respectively. Micro quantities of blood were introduced into two more chambers which were kept at a constant temperature. Subsequently they were vibrated while successively equilibrated with two (4 percent and 8 percent) CO_2 tensions from certified cylinders which were passed through humidifiers and temperature-controlled systems to the equilibrium chambers. The pH of the two samples was read after three minutes' equilibration. These two pH values and their corresponding CO_2 tensions were plotted. Using the actual pH as an entry, PCO_2 was derived from this line. Because of inherent inaccuracy of the method when applied to wild animals the PCO_2 in the later stages of the experiment was measured directly with a PCO_2 'E 5036' radiometer electrode. Similarly the PO_2 tensions were

measured with PO_2 'Clarke'-type 'E 5046' radiometer electrode.

Muscle pH

This was determined on a limited number of cases by measurements directly on the muscle fibres. A portable pH meter was used with a narrow probe. It possesses an enlarged scale sensitive to 0,01 pH units. Buffer solutions and distilled water were carried in plastic stoppered containers and corrections were made for the difference in temperature of the animal and the buffer solutions. The probe was inserted through a stab wound made in the skin after injection of local anaesthetic. The muscle fibres were separated by blunt dissection with a 'Spencer-Wells' haemostat.

Comparison of Methods to Determine Acid-Base Levels in Blood

Materials And Methods

A series of five tame blesbok were used. These animals had been kept under captive conditions at the Animal and Dairy Research Institute at Irene since birth and were therefore relatively tractable for handling and drawing of blood samples.

pH, PCO_2 and PO_2 levels were derived in three ways. By calculation from the Siggaard-Andersen Curve Nomogram; from direct measurements with a 'Severinghaus' PCO_2 electrode and also by calculation with an acid-base simulator using central venous, arterial and capillary blood.

RESULTS

FOR each animal, the result of each method was ranked in numerical order and Kendall's test of concordance applied. The formula for this test is discussed under the section on statistical methods in this Chapter. The actual results are tabulated in Chapter Three under the acid-base section. The resulting concordance coefficients for each parameter on arterial blood was as follows:

pH : W = 1,000***

PCO_2 : W = 0,92179***

PO_2 : W = 1,000***

The rank correlations were as follows:

pH : r_{12} = 0,841***
r_{13} = 1,000***
r_{23} = 0,841***

PO_2 : r_{12} = 1,000***
r_{13} = 1,000***
r_{23} = 1,000***

PCO_2 : r_{12} = 0,875***
r_{13} = 0,818***
r_{23} = 0,953***

Both the rank correlation coefficients and the concordance

coefficients are highly significant.

Discussion

All three methods are in highly significant concordance with the results on all three parameters measured on arterial blood. However, the results obtained on venous blood would appear unreliable with regard to PCO_2, although there is too little data for this to be proved statistically. pH and PO_2 measurements are identical for each method on venous blood, and can therefore be said to be in concordance.

Glutamic Oxaloacetic Transaminase and Glutamic Pyruvic Transaminase

Serum or plasma may be used for the determination of these enzymes although serum is more stable for certain periods of time (see section on stability tests in this Chapter). The procedure follows that described in the standard test kits ('Lange'), and is measured on a 'Lange LP 3' spectrophotometer.

CREATINE PHOSPHOKINASE AND LACTATE DEHYDROGENASE

SERUM or plasma may be used but see previous page. The procedure follows that described in the standard 'Boehringer Mannheim' test kits, and is measured on an 'LP 3' spectrophotometer.

LACTATE

PREPARATION of the freshly collected blood is necessary in the field immediately on collection. Perchloric acid 5,15 ml 70 percent (or 6,5 ml 60 percent) is first diluted with distilled water and made up to 100 ml. This is placed in ice-water prior to use. As the blood samples are taken, 1,0 ml of the diluted perchloric acid is pipetted into an empty 'Venoject' tube or 10 cc centrifuge tube. Using a 500 µl 'Socorex' pipette, 0,5 ml of the fresh sample is then pipetted into the perchloric acid and mixed well.

At the laboratory, the mixture is centrifuged for 10 minutes at 3 000 r.p.m. The supernatant fluid is drawn off and used for analysis. The procedure follows the standard 'Boehringer Mannheim' test, and is performed on the 'LP 3' spectrophotometer.

POTASSIUM

SERUM, free from haemolysis, is used for this test. The procedure follows the standard 'Haury' test method, and is performed on the 'LP 3' spectrophotometer.

UREA AND BLOOD UREA NITROGEN

FRESH serum is used for this test which follows the procedure of the standard 'Urea (Harnstoff)' test kit of 'Lange' and is measured on the 'LP 3' spectrophotometer.

CREATININE

FRESH serum is used for this test which follows the procedure of the standard 'Lange Kreatinin' test kits, and is measured on the 'LP 3' spectrophotometer.

SEPARATION OF MYOGLOBIN AND HAEMOGLOBIN

THE presence of both haemoglobin and myoglobin in the plasma of animals subjected to muscular stress was first determined by the well known method of salting out using two different concentrations of sodium sulphate. Since only small quantities of blood plasma were available, this method was

impracticable for quantitative determination. Subsequently a series of samples was subjected to 'Stärkeblock-Elektrophorese' (starch gel) according to the method of Marti (1961) and as described by Osterhoff, Ward-Cox and Pieterse (1971). Effective separation of myoglobin and haemoglobin both in serum samples as well as in artificially prepared mixtures of known composition of weights of crystalline myoglobin and haemoglobin was achieved. Approximate quantitative estimations were subsequently obtained colorimetrically.

This method was of sufficient exactitude to indicate the presence of both myoglobin and haemoglobin content and indicate the changes in proportion in relation to distance run and in animals subjected to capture by nets or restrained in the crush. To enable more exact routine estimations to be made as a standard determination of the degree of stress or possible preclinical or cryptic capture myopathy, polyacrylamide electrophoresis as described by Boulton and Huntsman (1971) was used. This method is accurate, readily subject to quantitative analysis, and furthermore the acrylamide gel with the separated protein bands may be stored for a period of months without deterioration so that large numbers of samples may be put through the analyser at the same time to preclude errors through variations in analysing procedure.

SELENIUM AND OTHER TRACE ELEMENT ESTIMATIONS

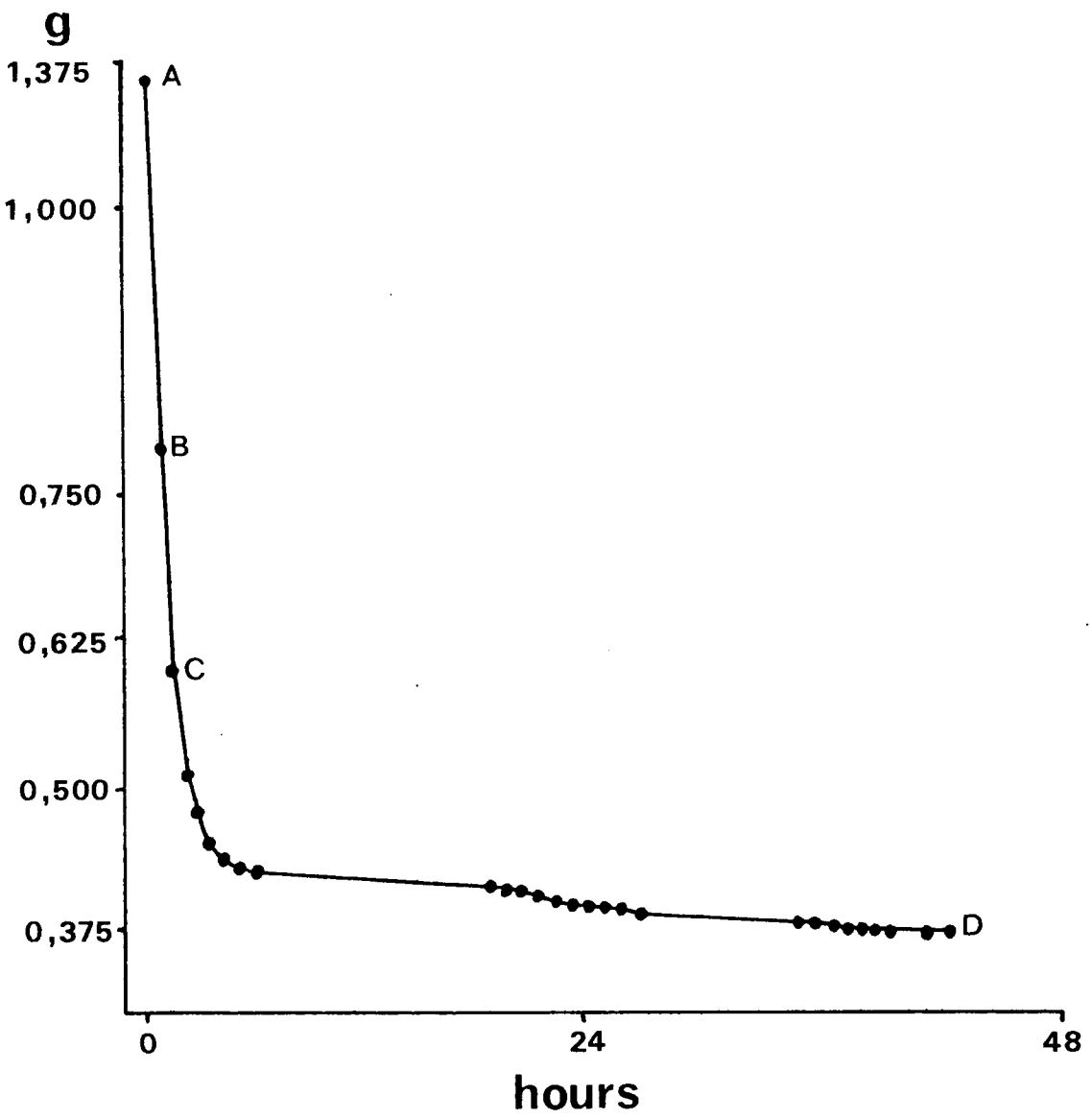
Drying Of Specimens

All estimations have been done on sections of liver tissue weighing approximately 30 g taken from presumed healthy animals cropped for various purposes unnconnected with this work, in several geographical locations, and preserved in formaline until needed.

Several grams (usually eight or nine grams) wet weight of liver was chopped into small pieces (about 3 mm^3 or less) to facilitate thorough and even drying. Precautions against contamination such as the meticulous cleaning of glassware, use of rubber gloves, etc., were taken. The chopped material was placed in a petri dish (uncovered) and thence into an oven pre-set for 108 °C, for 48 hours. Chemically cleaned bijoux bottles were put into the oven with the samples. Fig. 17 shows the curve of weight loss during the drying procedure. The samples were then placed into the bottles and the caps screwed on tightly.

Preparation Of The Standards

A stock solution analytical grade selenium dioxide was prepared at Pelindaba at the Atomic Energy Board, and diluted to contain 2 µg Se per ml of solution. Reference standards used were approximately 0,5 ml of the solution in polythene containers. The weights of the standard solu-



A = WEIGHT OF WET LIVER SAMPLE IMMEDIATELY PRIOR TO PUTTING INTO OVEN, AT 108 ^{0}C
B = WEIGHT OF SAMPLE ONE HOUR LATER
C = WEIGHT OF SAMPLE TWO HOURS LATER
D = WEIGHT OF SAMPLE FORTY HOURS LATER

Figure 17: Curve of weight loss during drying procedure of liver specimens over forty-eight hours.

tions were recorded and, after careful evaporation to dryness, each container was sealed.

'NBS Bovine Liver Standard Reference Material (SRM 1577)' was used as a reference standard containing 1,1 ± 0,1 µg selenium per gram of sample. Accurately weighed samples (~ 250 mg) of this standard were sealed in polythene containers.

IRRADIATION

THE samples and standards were irradiated separately and in fixed sequence for precisely 90 seconds. The relative values of the integrated neutron fluxes were determined for some samples and standards by using gold monitor samples. Irradations were done in the pneumatic facility of 'SAFARI-1', and 'ORR'-type reactor, in a thermal neutron flux of 2,89 $\times 10^{13}$ n cm^{-2} sec^{-1}. Westcott's epithermal index, r, for this irradiation position is 0,0087.

MEASUREMENT OF GAMMA ACTIVITY

GAMMA spectrometry of the irradiated samples and standards was performed after a lapse of exactly 20 seconds after each irradiation by placing them in a fixed position from the Ge(Li) detector. The detector used was a 50 cm^3 coaxial Ge(Li) diode (Princeton Gamma Tech.) connected to an uncooled 'TC 135M Tennelec' preamplifier. The output pulses were amplified by a 'TC 200 Tennelec' amplifier. Spectrum ana-

lysis was performed on an 'Intertechnique' 4 000-channel analyser (Model SA 44). The resolution of this counting system is 3 keV (full width at half maximum) for the 1 333-keV photopeak of ^{60}Co. Data for peak analysis were recorded on magnetic tapes which were processed by computer. Yule's (1968) smoothed first derivative method was applied to obtain the true peak counts under the photopeaks of interest.

CHEMICAL IMMOBILISATION

IMMOBILISATION of animals when required was carried out with the following mixtures. Etorphine and hyoscine hydrobromide for white rhinoceros; etorphine and acetylpromazine maleate for two free-living tsessebe at Percy Fyfe Nature Reserve. All other animals were immobilised with fentanyl, azaperone and xylazine hydrochloride. The dosage rates used were those according to Harthoorn (1976), those of the sable antelope being varied slightly as tabled in Appendix A.

STATISTICAL METHODS

THE significance of differences between various sets of data obtained before and after the various procedures were carried out was determined with Student's *t*-test for paired values (Steele and Torrie 1960). This test was used for experiments such as comparing haematocrit values in eland

from one reserve to the changes that occurred after relocation; differences in levels of various parameters before forced exercise and afterwards; the rise or fall in various parameters after exercise or after treatment. This test was used also in comparing enzyme levels from one chase to those of a second or subsequent chase, as well as the difference between stress after transport and stress after forced exercise. Similar parameters were also tested in this way, such as the comparison of muscle pH with blood pH. In addition, this test was used as a basis for testing stability of serum and plasma for analytical purposes, especially for stability of blood enzymes.

Where a test was not significant, 'not significant' was stated, and usually fell beyond the limits of the 10 percent significance level, or was not significant at any reasonable level.

Unless stated, all tests were made on a two-sided basis, i.e. the result was not expected to lie in one direction or another. Where the direction of the result was known, a one-sided test was performed and is stated as such.

Where the sample size was particularly small, and where two sets of data had to be compared as described for the *t*-test, the Wilcoxon matched pairs signed rank test was used (Steele and Torrie 1960). This was used especially in the determination of the efficacy of therapeutic substances, where treated and untreated groups were compared for signifi-

cance.

There were several instances where straightforward correlation r was made between unlike parameters. For this, the correlation coefficient was measured. Both one-sided and two-sided tests were made. Most cases included correlations such as that between body temperature and distance run; blood pressure and minutes after exercise; respiratory rates with speed of chase; pH, PCO_2, PO_2, standard bicarbonate and base excess with distance run; various trace elements with one another; haemoglobin and myoglobin in plasma with distance run; lactate with pH. Other correlations were measured between like parameters, but under varying circumstances, e.g. body temperature changes over a period of time, haematocrit values before and after exercise, as well as over a period of time after, say, immobilisation.

Correlation coefficients were also obtained to determine positive or negative correlations. Certain data underwent partial correlation analysis where three or more factors were being determined.

Both linear and non-linear regressions and correlations were calculated. The linear regressions were determined particularly in the cases where significant differences from zero (the X-axis) had to be tested, for example, to see whether there was significant change in a certain parameter over a period of time. If the regression was insignificant, the regression line was taken as constant, and

from this an idea could be obtained of possible normal or 'resting' values. For example, when several enzyme values were plotted over a period of up to four months, and the slope was negligible in the context of the experiment, the consistency of the values was an indication of establishing so-called normal values. In another experiment, the difference between the slopes of males and females after exercise for some parameters was measured, and the linear regression plotted. In many cases, the *t*-test for testing the sign of a regression coefficient was applied and compared to the critical values for significance (Owen 1962).

Non-linear regression curves were fitted to data where the line of values plotted resembled a parabola of the formula $\log Y = a + b \log X$. This applied particularly well in the case of the rate of recovery of pH values in exercised zebra and blue wildebeest before and after treatment.

Kendall's test of concordance was used in cases where a certain number of methods were used to determine the same parameter. This method of rank correlation determined the reliability of the results using all three methods (Downie and Heath 1974).

In order to determine the correlation of one month in one year with another month the following year with regard to selenium levels in liver, Spearman's coefficient of rank correlation was used (Steele and Torrie 1960). This was used to indicate some consistency in seasonal variation, or lack

of it.

Throughout the text asterisks are used to denote the following:

* = significant at the 10 percent level of significance. This means that for every test, 10 percent of the time there will be an error. Ten percent is therefore regarded as not particularly significant in the context of the experiment.

** = significant at the 5 percent level of significance. This is regarded as reasonably significant in the context of the experiment.

*** = significant at the 1 percent level of significance. This is regarded as highly significant in the context of the experiment.

Where asterisks are not given, the degree of significance is stated, e.g. significant at 2 percent, or between 10 and 25 percent, etc.

CHAPTER THREE

NORMAL VALUES

INTRODUCTION

NORMAL values of blood constituents, the electrocardiogram, etc., are notoriously difficult to obtain in domestic animals. Comparable values in wild and captive wild animals have hardly been studied.

It has been attempted to obtain resting values of the principal parameters which are being dealt with as criteria of abnormality. In this there has been a measure of success insofar as resting ranges appear to have been obtained.

As examples, there are the steady levels of GOT, GPT and CPK in trained eland. It was found that if the diesel pump was always switched on as at feeding time, the animals could be blindfolded and sampled with very little effort or resistance on their part.

Even with newly captured wild zebra and wildebeest, the sampling routine eventually became accepted, even though the animals had to roped for this operation. The various enzymes fell to low steady levels.

The belief that normal values were being recorded was re-

inforced by the similarity between the resting levels and those of domestic animals, even to those of man.

BLOOD ENZYMES

Materials And Methods

To establish resting values of blood enzymes, eight eland were used. These animals were trained to a sampling routine so that regular blood samples could be obtained with a minimum of disturbance every two to three weeks in the manner described in Chapter Two. Three zebra were also used while in pens at Skukuza over a period of three months. Seven white rhinoceros were captured by drug immobilisation in the Hluhluwe/Umfolozi complex and peripheral blood taken as described in Chapter Two.

Results

About one month after arrival at Percy Fyfe Nature Reserve, the enzyme levels in the eland settled down to a steady level which was very similar to the normal levels in man. These enzymes remained at steady levels with very little fluctuation (Fig. 18).

Pearson's product moment correlation was used to calculate the correlation of values over this period of about two-and-a-half months, and the regression was tested for constancy,

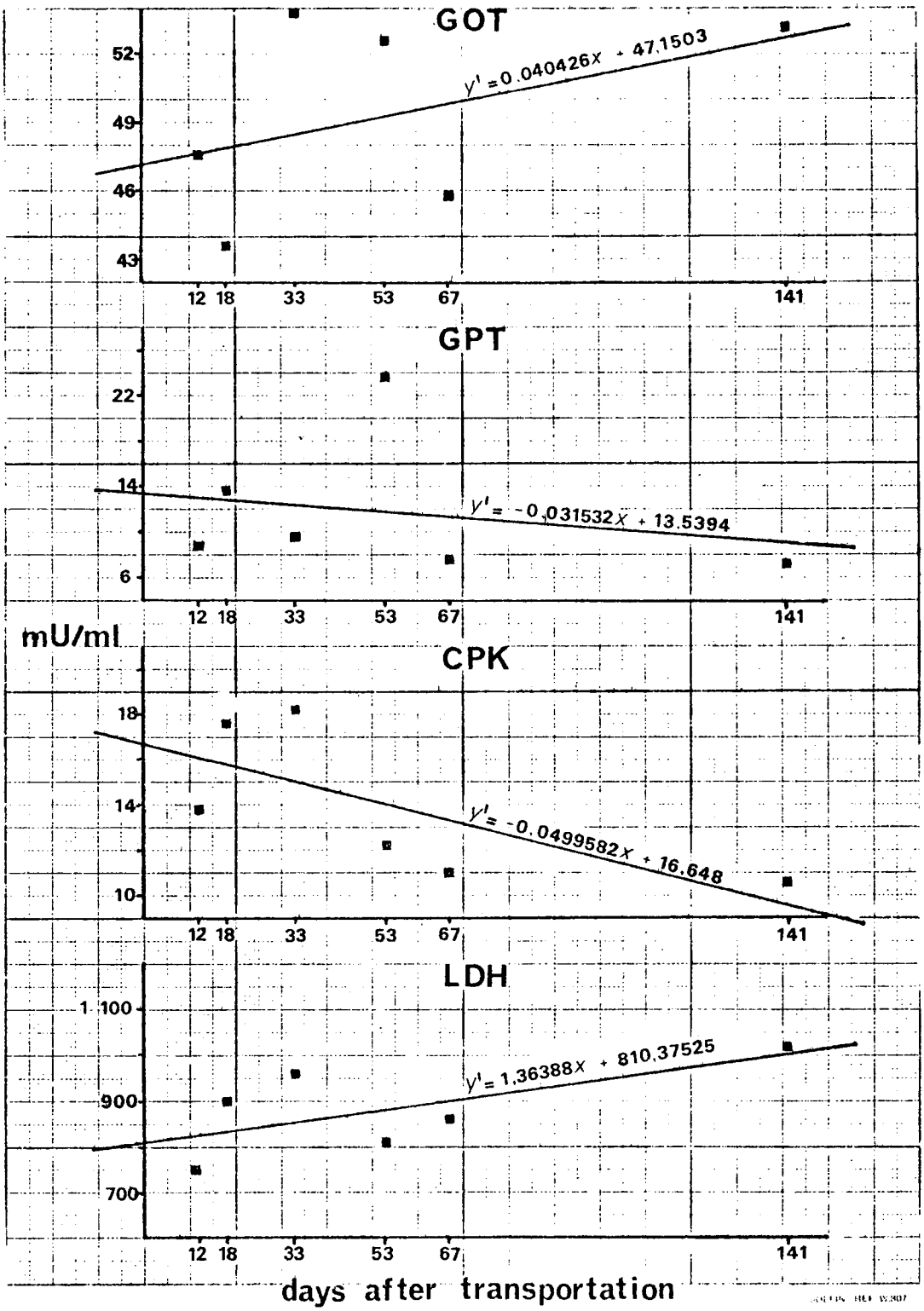


Figure 18: Enzyme levels in eland at Percy Fyfe over a period of four-and-a-half months.

i.e. to test if the slope was significantly different from zero. The purpose of this test was to establish the so-called normals, or resting values of the enzymes. The correlation coefficients are as follows:

GOT : r_{xy} = 0,453568
GPT : r_{xy} = -0,235297
CPK : r_{xy} = -0,7008492
LDH : r_{xy} = 0,6528904

} (n = 6) not significant at any reasonable level

The regression equations and t-test for significance of slope are as follows:

GOT : $y' = 0,040426x + 47,1503$ $t_4 = -1,02$
GPT : $y' = -0,03153x + 13,5394$ $t_4 = -0,48$
CPK : $y' = -0,04996x + 16,648$ $t_4 = -1,97$
LDH : $y' = 1,36388x + 810,3753$ $t_4 = 1,72$

} not significant at any reasonable level

The numbers of zebra were insufficient to give statistical results. Apparent resting values were obtained for GPT, CPK and LDH (Fig. 19).

The results of the white rhinoceros are shown in Table 3, and compared closely with those obtained from other animals as above and those for domestic sheep and horses.

Discussion

The regression of values in the eland enzymes over a period of time are shown to be constant, which indicates that these values may be taken to be expected resting values, or could

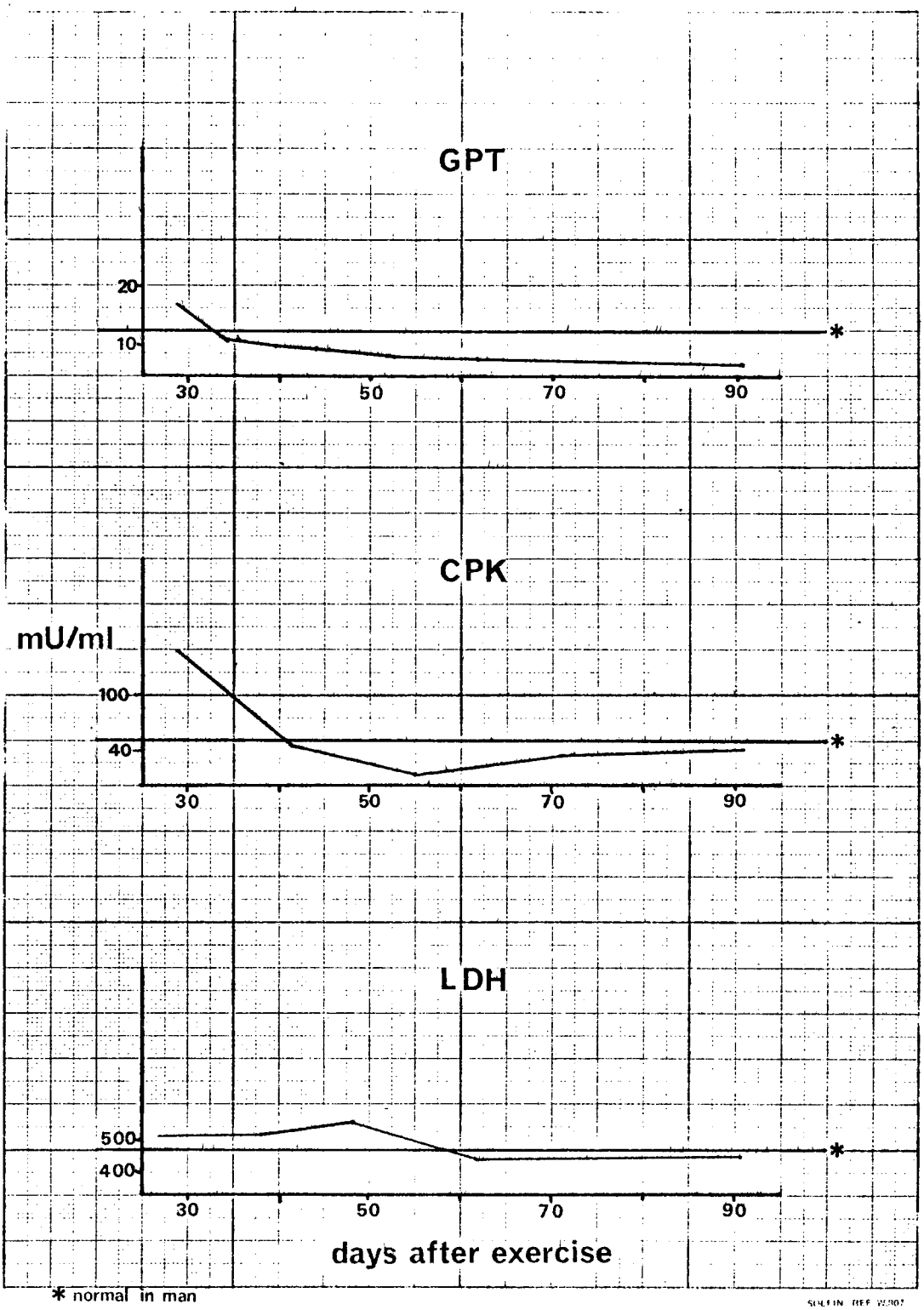


Figure 19: Enzyme levels in zebra in pens at Skukuza over a period of three months.

Table 3: Enzyme levels in square-lipped rhinoceros immobilised at Umfolozi Game Reserve, compared with known normals.

	GOT	GPT	CPK	LDH
Normals				
man	< 12	< 12	< 50	< 195
horse†	40 - 100	5 - 25	8 - 40	250 - 460
sheep†	45 - 80	12 - 34	8 - 60	450 - 690
Animal no.				
1	85	4	16	334
2	17	7	24	477
3	15	8	16	305
4	15	4	16	449
5	14	3	32	372
6	36	6	87	525
7	78	7	83	210
Averages of the group	37	6	39	382

† from the Department of Medicine, Faculty of Veterinary Science (Courtesy Professor K. van der Walt)

be taken as a basis for so-called normal values.

As the rhinoceros were subjected to some disturbance prior to and during immobilisation, these results cannot be classified as resting values, but appear close to those which would be obtained had the immobilisation been effected under ideal conditions as with the two tsessebe described below.

HAEMATOCRIT

MATERIALS AND METHODS

EIGHT eland were sampled as described above, using a peripheral vein and sampling without stasis.

RESULTS

THE regression line of values was shown to be constant for the two-and-a-half month period at Percy Fyfe (Fig. 20).

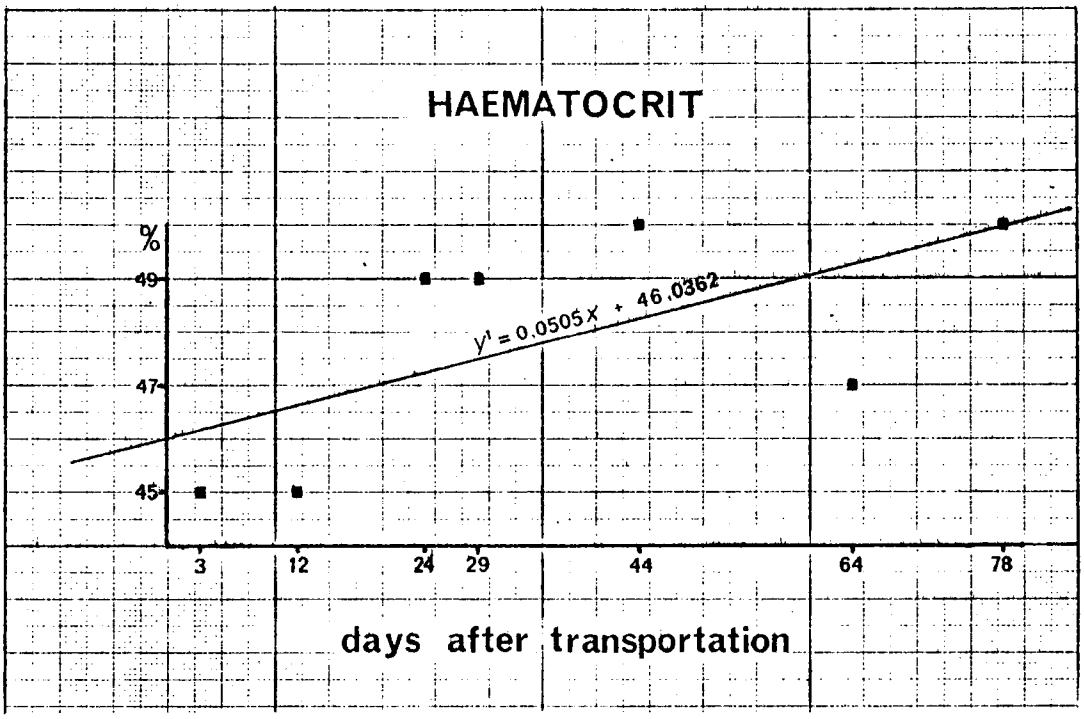


Figure 20: Haematocrit levels in eland at Percy Fyfe Nature Reserve over a period of two-and-a-half months.

The regression equation is as follows:

$y' = 0{,}0505x + 46{,}0362 \quad (n = 7)$

This slope was calculated to be not significantly different.

Discussion

The values taken at Percy Fyfe can be regarded as constant, as the regression was not significant.

LACTATE

Materials And Methods

Blood samples for lactate were taken from eight eland and subjected to immediate processing as described in Chapter Two.

Results

The regression equation was calculated for the values over a period of four-and-a-half months as follows (Fig. 21):

$y' = -0,0822x + 29,676 \quad (n = 7)$

The significance of the slope was calculated as $t_5 = -3,338^{**}$.

Discussion

There is a significant slope in values from after transportation to before exercise nearly five months later, although the points on the graph after 12 days after transport show a general settling down of values to normal levels (normal in man is 16 to 22 mg percent). Strictly speaking, resting values may not be statistically significantly established

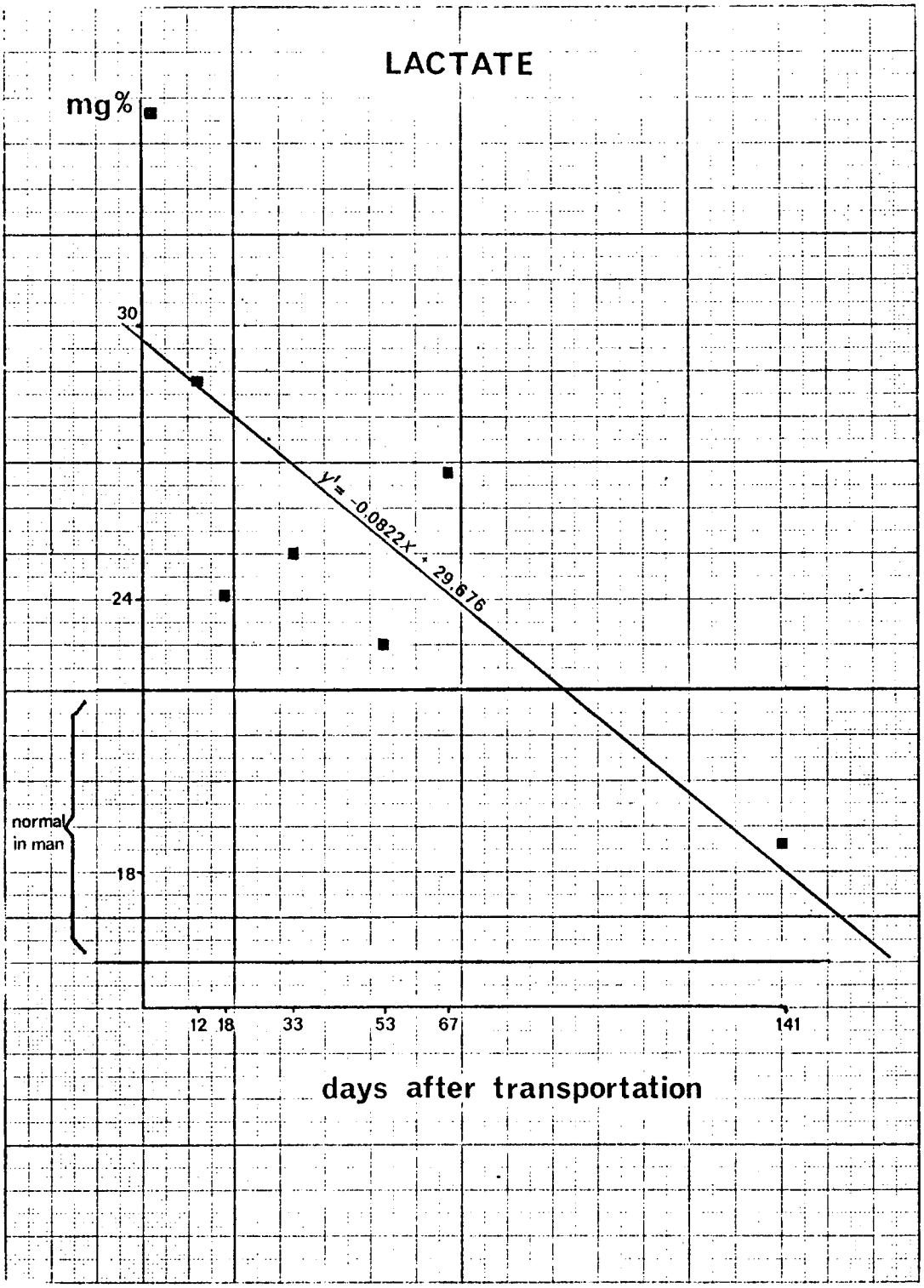


Figure 21: Lactate levels in eland at Percy Fyfe Nature Reserve over a period of four-and-a-half months.

from the data available.

BLOOD GASES AND ACID-BASE VALUES

Materials And Methods

Two male tsessebe were lightly immobilised and subjected to puncture of a superficial artery as described in Chapter Two. Five tame blesbok were sampled in a similar way.

Results

The results of the tsessebe are shown in Fig. 22 and those of the five blesbok in Table 4.

Discussion

The values obtained from the two tsessebe and from the five tame blesbok are identical to the normal range of values accepted for man, and very similar to the values obtained from aortic puncture in animals after bicarbonate infusion (Chapter Four).

Resting values of blood gases and pH obtained from arterial cannulas in wild animals cannot be regarded as normal on account of the continued restraint necessary especially in wildebeest and zebra. Even in zebra, however, the values for PO_2 and PCO_2 approach the normals for the

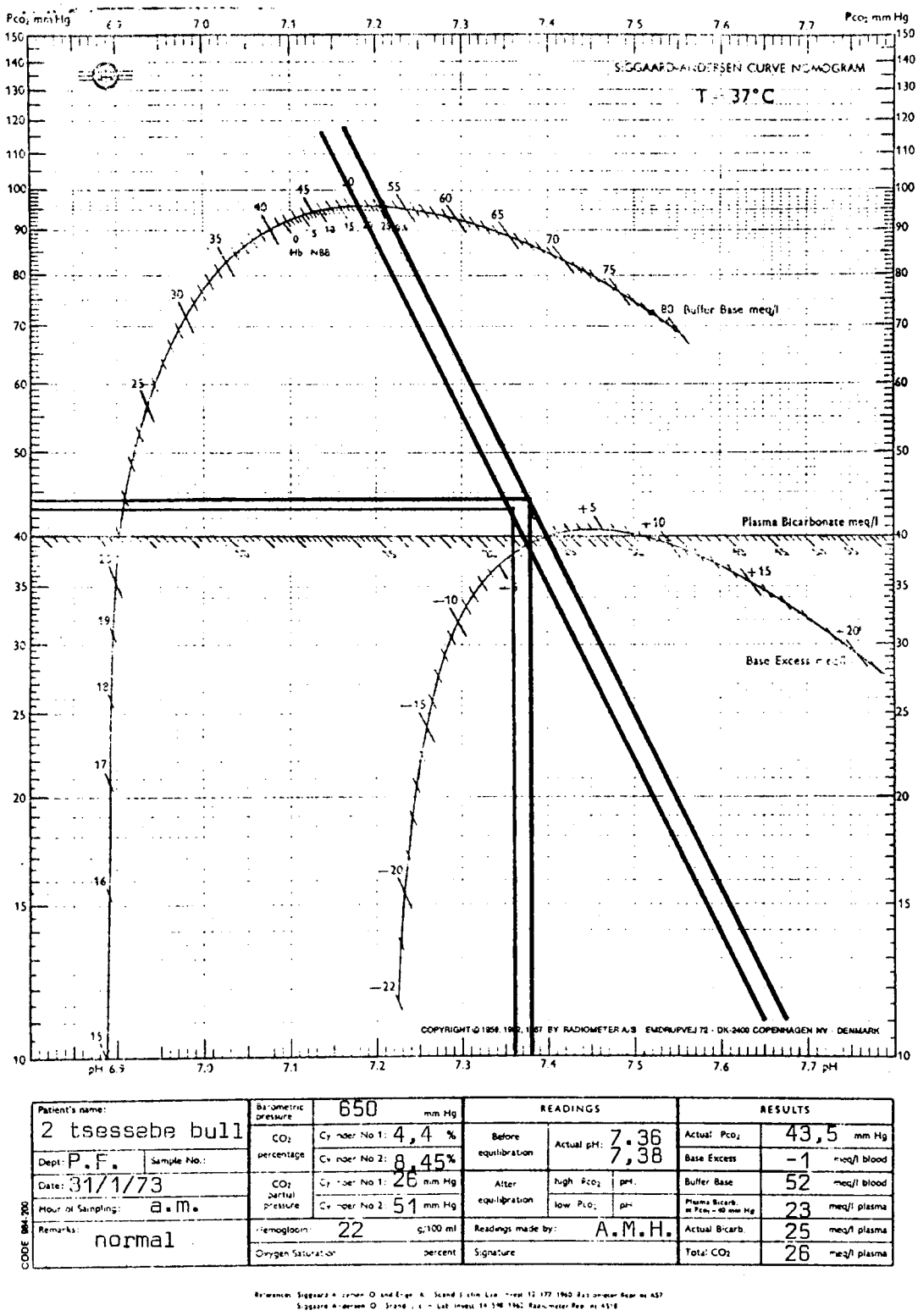


Figure 22: Two male tsessebe immobilised with etorphine/acepromazine mixture - acid-base levels.

Table 4: Acid-base levels in five tame blesbok held at A.D. R.I. at Irene using three methods.

	Astrup			Simulated	Direct		
	art.	ven.	cap.	art.	art.	ven.	cap.
1							
pH	7,444	7,393	7,430	7,420	7,444	7,393	7,430
PCO_2	36,9	43,3	-	36,9	38,1	47,9	35,0
std. bicarb.	24,7	25,0	-	24,7	-	-	-
base excess	+0,02	+0,05	-	0,00	-	-	-
actual bicarb.	24,6	27,4	-	24,5	-	-	-
buffer base	43,5	43,9	-	43,5	-	-	-
total CO_2	25,7	28,6	-	26,6	-	-	-
PO_2	62,0	43,0	62,0	-	62,0	43,0	62,0
2							
pH	7,412	7,374	7,440	7,410	7,412	7,374	7,440
PCO_2	32,3	37,6	-	32,3	34,0	44,3	28,9
std. bicarb.	21,0	22,5	-	21,0	-	-	-
base excess	-4,01	-2,37	-	-4,01	-	-	-
actual bicarb.	24,5	24,0	-	19,4	-	-	-
buffer base	40,8	54,0	-	40,8	-	-	-
total CO_2	25,4	25,1	-	22,0	-	-	-
PO_2	62,0	35,0	clot	-	62,0	35,0	clot
11							
pH	7,440	-	-	7,440	7,440	-	-
PCO_2	31,8	-	-	30,5	30,0	-	-
std. bicarb.	21,8	-	-	22,0	-	-	-
base excess	-2,3	-	-	-2,2	-	-	-
actual bicarb.	20,4	-	-	20,0	-	-	-
buffer base	39,8	-	-	39,8	-	-	-
total CO_2	21,5	-	-	22,0	-	-	-
PO_2	104,5	-	-	104,5	104,5	-	-
14							
pH	7,400	-	-	7,400	7,400	-	-
PCO_2	33,5	-	-	35,1	35,1	-	-
std. bicarb.	23,0	-	-	22,5	-	-	-
base excess	-1,2	-	-	-2,0	-	-	-
actual bicarb.	19,5	-	-	20,0	-	-	-
buffer base	59,8	-	-	59,8	-	-	-
total CO_2	20,5	-	-	22,5	-	-	-
PO_2	101,5	-	-	101,5	101,5	-	-
20							
pH	7,437	-	-	7,437	7,437	-	-
PCO_2	35,3	-	-	34,0	34,0	-	-
std. bicarb.	24,7	-	-	23,0	-	-	-
base excess	+1,0	-	-	0,0	-	-	-
actual bicarb,	23,0	-	-	22,2	-	-	-
buffer base	54,7	-	-	54,7	-	-	-
total CO_2	24,1	-	-	24,5	-	-	-
PO_2	101,5	-	-	101,5	-	-	-

art. = arterial, ven. = venous, cap. = capillary

horse (Littlejohn 1975, Littlejohn and van Heerden 1975).

BODY TEMPERATURE, PULSE AND RESPIRATORY RATES

NORMALS for body (core) temperature have been established for several species of wild ungulates including hartebeest, zebra, wildebeest and eland (Bligh and Harthoorn 1965, Harthoorn and McGinnis 1971) using biotelemetric techniques on a continuous basis. There was a close correlation between core and rectal temperature.

Heart and respiratory rates over periods of three to four days were determined for eland and hartebeest as compared to Boran cattle by Harthoorn, Finch, Hopcraft and McGinnis (1970). Variations in body temperature and respiration as a result of exercise was determined by Harthoorn and McGinnis (1971).

CHAPTER FOUR

ACID-BASE BALANCE AND BLOOD GASES

INTRODUCTION

Blood pH

A role played by blood pH in locomotory stress has long been recognised. The assumption, based on human experiences was, however, that the symptoms of acidaemia were due primarily to hypoventilation, hypoxaemia and hypercapnoea. The lack of oxygen in relation to the body's demands under strenuous exercise resulted in incomplete combustion of glycogen and the consequent formation of lactic acid. This acidity was, however, enhanced by a retention of carbon dioxide and consequent formation of carbonic acid. This situation is reversed fairly rapidly after the termination of exercise when the so-called oxygen debt is liquidated. The blood pH changes in man under conditions of exercise are relatively slight, and most sources still give a pH of 7 as lethal unless corrected. The normal range for the horse is given as 7,2 to 7,55; for cattle and sheep 7,27 to 7,49 and 7,32 to 7,54 respectively, with mean values for these two species as 7,38 and 7,44 (Swenson 1970). Slightly lower pH changes are found in conditions of diabetic coma. Other conditions that cause acidosis in man are loss of chlorine ions which may result

from vomiting or diarrhoea; an excess of potassium ions from intracellular sources such as blood or muscle fibres, a deficiency of aldosterone resulting in reduced sodium ion reabsorption from the kidney and subsequent reduction of hydrogen ion secretion, and lastly due to uraemic conditions caused by a failure of the kidney to rid the body of the normal metabolic acids (Swenson 1970).

An experiment entailing forced exercise was conducted on blesbok over varying distances for determination of blood pH,etc. Problems experienced while chasing on the broken ground at Percy Fyfe, the paucity of animals, as well as the need to investigate other species dictated a further series of experiments to be conducted in the Kruger National Park starting with zebra and blue wildebeest. The flat areas of Tshokwane, Satara, Lindanda, Mtomene and later Mananga proved ideal for short rapid chases as here the animals were easily followed and were usually unable to escape into dongas and patches of scrub as in more broken country. Here it was possible to confirm the findings that the intense sharp chase was inclined to be the most harmful, and that the pejorative effects in these cases are mediated apparently entirely through extremely low blood pH values of 6,5 or less. This is 50 points less (on a logarithmic scale) than the normally considered minimum of 7,0. It should be noted that the comparison that is being made is not solely between a short rapid chase and a prolonged slow chase. It would appear (although not necessarily to be recommended) that an intensive chase carried out after or subsequent to a prelim-

inary chase where the animal is not fully extended, is less deleterious in its action and less inclined to be fatal than a similar short chase carried out from a standing start.

Muscle pH

A small series of measurements have been made directly on the muscle fibres of acidotic zebra. The primary object was to investigate the possibility of the muscle fibres themselves exhibiting a pH lower than that of the arterial and venous blood (see below).

MATERIALS AND METHODS

Blesbok

Eighteen blesbok which resided in 600 ha (700 morgen) paddocks at Percy Fyfe Nature Reserve were used. They were subjected to forced exercise by chasing in groups as described in Chapter Two. The environmental temperature at the time of chasing was 21 to 23 °C. The groups of animals fell into three categories and these are given in Table 5. The distances travelled could not be predetermined, so that the precise distance covered by each of the animals is uncertain as the individual animals do not run directly in front of the trucks, nor do the groups of antelope show cohesion. At the end of the chase the animals were herded into a small circular paddock from which they could readily

be caught, or else driven into nets.

Table 5: Distances and times run for three groups of eighteen blesbok chased at Percy Fyfe Nature Reserve.

group	approx. distance run (km)	time (min.)
1	2	5 - 10
2	4	10 - 16
3	6 - 10+	10 - 30

Arterial samples were drawn by direct puncture of a peripheral artery and analysed with a radiometer and pH meter.

Zebra And Blue Wildebeest

The methods employed were predominantly the same as those described for blesbok capture with one notable difference. The animals were not chased into nets but overhauled and seized by ears or tail. This appeared to cause far greater stress as the animals were extended to the full while trying to escape from the pursuing vehicle. These animals were chased individually as compared to herding groups into nets or enclosures where no animals were singled out and followed exclusively thus being driven to their maximum speed.

Infusion To Counteract Acidaemia

A mixture of sodium bicarbonate ($NaHCO_3$) in 0,9 percent

physiological saline or 'Normosol' solution was infused within an hour after capture in those animals randomly selected for treatment. No infusion was given to control groups. The calculation for zebra and wildebeest is as follows:

FORMULA

$$BW \times -BE \times 0,3$$

BW = Body weight in kg

-BE = Negative base excess

0,3 = Arbitrary factor at altitude of ± 2 000 m

At sea level there is more bicarbonate and this factor is 0,2.

Thus 0,3 × 2 500 m-equiv./l $NaHCO_3$

85 g/l = 8,5 percent which gives 1 m-equiv./ml or 1 000 m-equiv./l

Infusion of zebra and wildebeest show that a pH shift from approximately 6,5 to 7,4 is obtained from the infusion of 1 000 m-equiv. $NaHCO_3$.

The composition of 'Normosol' is as follows:

'Normosol^R -R' pH 7,4

Each 1 000 ml contains:

Sodium chloride B.P.	5,26 g
Sodium acetate B.P.	3,68 g
Sodium gluconate	5,02 g
Potassium chloride B.P.	0,37 g
Magnesium chloride B.P.	0,30 g

Approximate m-equiv./l:	
Sodium	140
Potassium	5
Magnesium	3
total cations	148
Chloride	98
Acetate	27
Gluconate	23
total anions	148

A total of twenty-five zebra and wildebeest were used in the acid-base experiments, fourteen of which received infusion therapy. Particulars of these animals are given in Table 6.

Muscle pH

A portable pH meter was used with narrow probe as described in Chapter Two.

RESULTS

Blesbok

Bicarbonate

Levels of bicarbonate were low in all groups after the compl-

Table 6: Particulars of zebra and blue wildebeest exercised in the Kruger National Park.

species	sex	age (yr)	est. wgt (kg)	distance chased (km)	treated	survived
zebra						
	F	1	200	1,0	no	no
	M	2	250	5,0	no	no
	M	1	200	2,0	no	no
	F	2	250	1,3	yes	yes
	M	2	250	2,1	yes	yes
	M	2	250	3,3	yes	yes
	F	2	160	0,9	yes	yes
	M	2,5	180	3,1	yes	yes
	F	6 mo.	160	1,6	yes	yes
	F	2	250	2,0	yes	yes
	M	adult	400	2,5	no	no
	M	9 mo.	90	3,3	yes	yes
	M	4 mo.	70	1,7	no	yes†
	M	1	135	2,0	yes	yes
wildebeest						
	F	4 mo.	35	3,2	no	yes
	M	3 mo.	25	3,8	no	yes
	F	6 mo.	45	2,8	no	yes
	M	1	115	7,2	no	no
	M	1	115	2,5	no	no
	F	1	115	2,5	yes	yes
	M	2,5	135	2,6	yes	yes
	M	2,5	160	3,0	yes	yes
	F	3 mo.	70	1,3	yes	yes
	F	4 mo.	70	4,0	yes	yes
	M	adult	270	2,2	no	yes††

† did not follow this one up - may have survived - not very stressed

†† started to recover before infusion - not very stressed

etion of the run. The lower values immediately after exercise were 10 m-equiv. with an average of under 15 m-equiv. (Fig. 23). Where samples were taken at 10 minute intervals, bicarbonate levels showed a small rise after 20 min. Most animals showed normal levels after three hours.

Base Excess

The pattern of recovery of the base excess is similar to that of bicarbonate levels. Levels rose from an average of -15 m-equiv. to zero over a period of approximately three hours with the lower value rising from -18 to -5 over that time (Fig. 24). The lower range represents animals of Group 3 that ran for the longest distances. Where samples were taken at 10 min. intervals, the base excess showed steady levels for the first 20 min. after which it commenced to rise.

Hydrogen Ion Concentration

The hydrogen ion concentration from samples taken at 0, 3 and 6 hours after exercise shows good recovery after a period of three hours. In samples taken at frequent intervals in small groups of animals the pH shows a drop during the first 10 min. after capture followed by a rise in 20 min. (Fig. 25). Minimum pH levels were attained by the group that ran the shortest distance, although in Group 3 the minimum pH value was still 7,26. The highest pH value in capture was found in Group 3 that ran the furthest distance of 10 km and in one exhausted animal in Group 2.

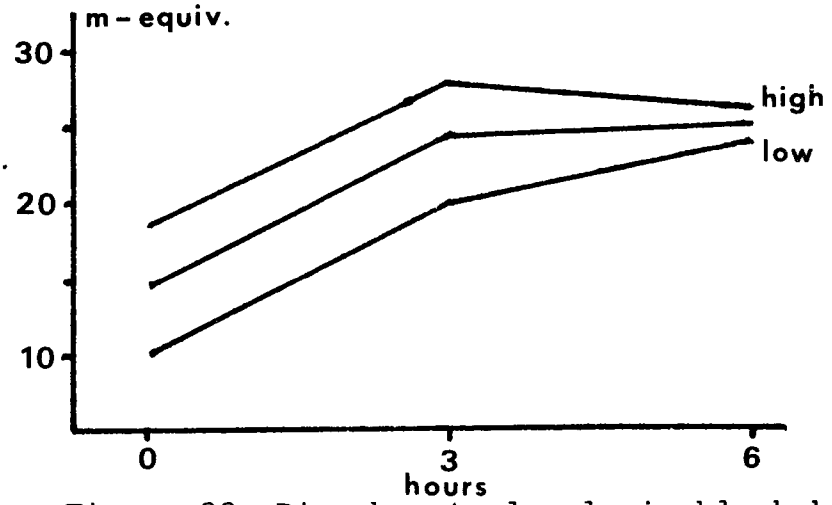


Figure 23: Bicarbonate levels in blesbok immediately, three and six hours after exercise.

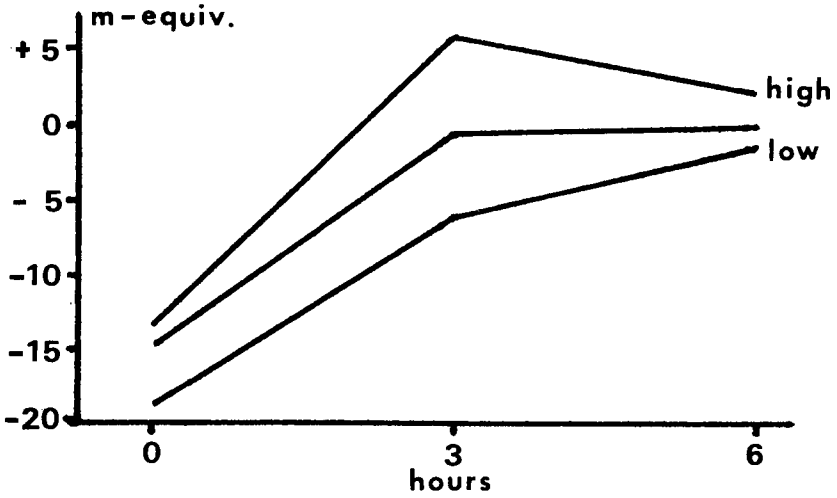


Figure 24: Base excess levels in blesbok immediately, three and six hours after exercise.

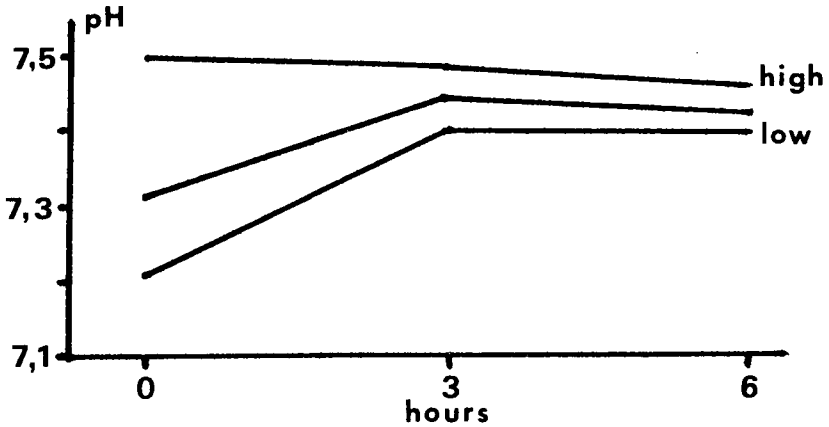


Figure 25: pH levels in blesbok immediately, three and six hours after capture.

PO_2 And PCO_2

Values for PO_2 showed a wide spread with the indication that compensation for blood gases is extremely rapid, and that there is evidence of hyperventilation. A degree of compensation between capture and the actual taking of the sample (within 5 minutes) is possible. There was in most cases an early and rapid fall in PO_2 levels during the first 20 min. after capture from levels of about 95 to 90 mm Hg to 75 mm Hg. This corresponded to a reduction of excitement at the end of the chase, and also to a change in respiratory pattern and thermal panting. There were also considerable differences in arterial PO_2 levels that do not appear to correspond with any one group. After an initial fall, values appeared to stabilise towards six hours (Fig. 26). The PO_2 results are, however, subject to considerable artifact while the animals responded by hyperventilation immediately on renewed handling, and there may well have been a greater reaction at six hours than at three when the animals were still suffering from exhaustion. It is probable that little value can be attached to PO_2 values in wild animals taken at times other than immediately after capture, unless an artery is catheterised.

The levels of PCO_2 showed an overall rise over three hours, again indicating a degree of overventilation. Moreover, a more frequent sampling on some of the animals showed that there was a fall in PCO_2 levels over the first 10 min. from about 20 - 25, to 13 - 15 mm Hg after which there was a steady rise to normal levels (Fig. 27). Here again, however,

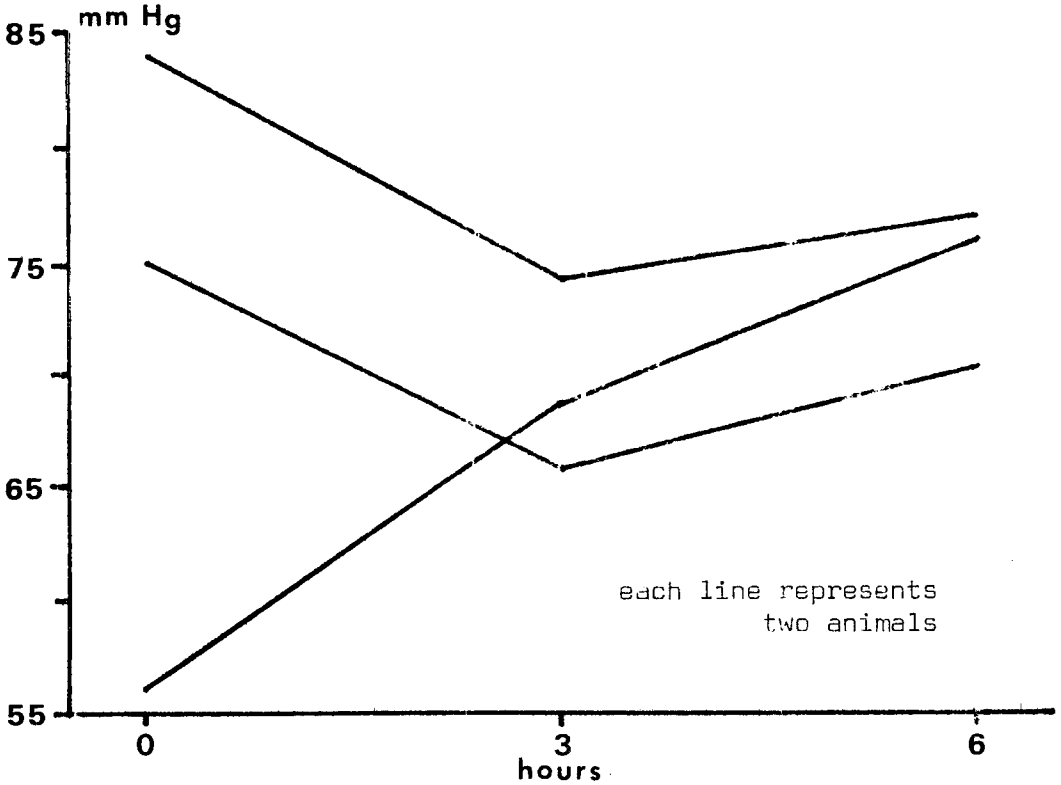


Figure 26: PO_2 pressures in blesbok immediately, three and six hours after exercise.

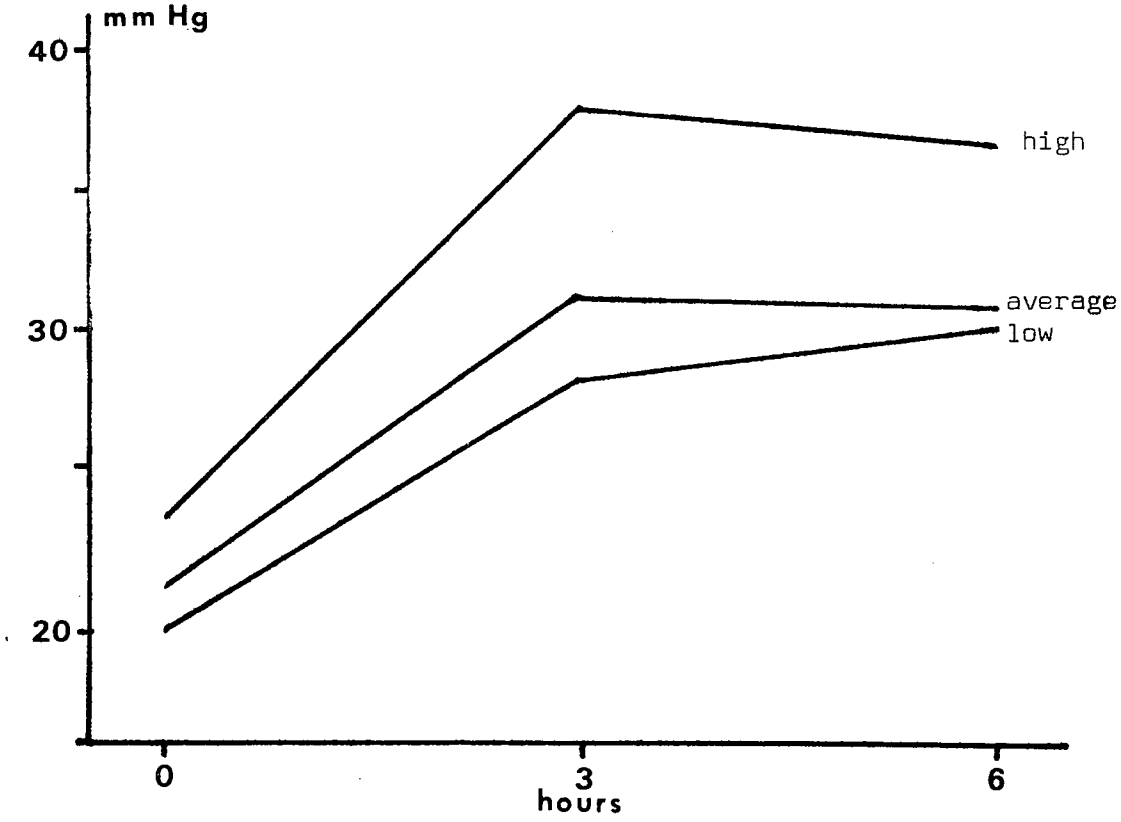


Figure 27: PCO_2 pressures in blesbok immediately, three and six hours after exercise.

arterial samples for PCO_2 analysis may be subject to similar disadvantages as for PO_2 estimations. The correlation coefficients were calculated on five acid-base parameters and distance run. The results were as follows:

Distance run correlated with:

pH:	$r = 0,1613$ $(n = 18)$	not significant at the 10 percent level
PCO_2:	$r = -0,208$ $(n = 15)$	
std. bic.:	$r = 0,1759$ $(n = 18)$	
base ex.:	$r = 0,2141$ $(n = 18)$	
PO_2:	$r = 0,3645$ $(n = 17)$	

The closest correlation, albeit not significant, is with PO_2.

Zebra In 1973

After capture the animals were restrained in lateral recumbency initially for 30 min. During these 30 min., marked deterioration of all animals was evident. Heart rates rose to levels of 380 beats per minute, and respiration remained rapid despite the complete rest. The values are shown in Fig. 28. The pulse became progressively more 'thready' and the heart beat muffled. There was little attempt at movement or struggling.

The muscles became gradually stiffer and apparently painful. After 30 min., the zebra were almost incapable of rising. When placed on their feet they were disinclined to move and occasionally unable to stand. The legs were

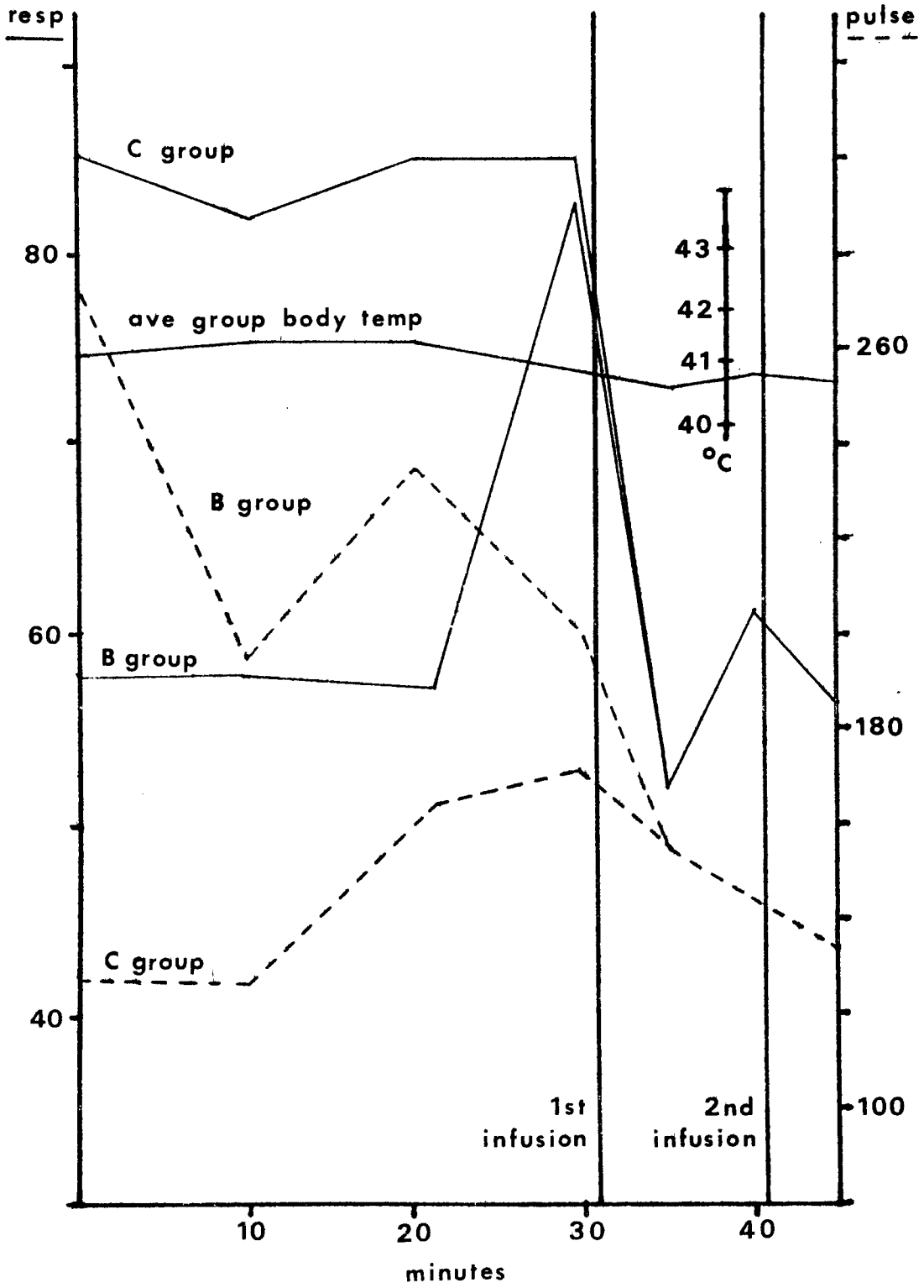


Figure 28: Pulse, respiration and body temperature in zebra before and after treatment with bicarbonate infusion.

held stiffly, and the head low.

Six animals were taken to pens at Skukuza. On arrival they drank but remained standing stiffly, and would only take a few steps if pushed. Pulmonary oedema developed and death occurred from 30 min. to 12 hours in untreated animals.

The pH as measured immediately after capture showed a further decrease in a few animals in the subsequent 30 min. showing inability to compensate by CO_2 elimination.

The captured zebra that survived were released one month later for recapture. One of these zebra (1 of Group C) showed a marked acidosis (pH 7,041) at the time of release representing the stress of handling, crating and transport over 70 km. This indicates that wild animals may become severely acidotic from causes unconnected with intensive exercise (Table 7). Respiration remained rapid and became progressively shallower.

Estimation of enzyme levels showed no significant rise by the time of capture. In those animals that were treated and as a result survived, steep rises in GOT and CPK levels indicate muscle damage (Chapter Ten).

Post mortem examination showed the most marked lesions to be extensive areas of pale degenerated muscle, interspersed with haemorrhages, thus fitting in with the accepted picture

Table 7: Blood pH levels in zebra before and after treatment with bicarbonate.

zebra		pH on capture	pH pre infusion (30 min. later)	pH post infusion (500 m-eq.)	pH post infusion (1 000 m-eq.)	survival
Group A	1	6,50	6,45	-	-	died 9 hours later
	2	6,50	-	-	-	died 30 minutes later
	3	6,70	6,55	-	-	died 12 hours later
Group B	1	6,85	6,77	7,07	7,10	yes
	2	6,70	6,68	6,92	7,17	yes
	3	6,80	6,67	7,24	7,42	yes
Group C	1	7,01	6,86	7,21	7,32	yes
	2	6,97	6,79	7,13	7,30	yes
	3	6,94	6,78	7,22	7,40	yes

Group A = untreated animals

Groups B and C = these groups comprise the same animals on the first and second chase

of capture myopathy. Necrosis of the cardiac musculature, pulmonary oedema and general cyanosis, also observed, may indicate the eventual cause of death.

Zebra And Wildebeest In 1974

The results of PCO_2 estimations appear at first sight to

be paradoxical. During the first half hour after capture, the PCO_2 levels *show a rise* from about 23 to 32 mm Hg pressure. This trend is opposite to that of the PO_2 (the PO_2 falls) and is ascribed mainly to the hyperventilation at the time of capture.

The results in the wildebeest differ from those obtained in zebra in that a considerable compensation, probably of respiratory origin, occurs between capture and infusion. This improvement is not unexpected in view of the resistance that the adult blue wildebeest are stated to have to capture myopathy (Young, pers. comm.†).

After infusion, the PCO_2 levels rose further, and this is associated with a drop of PO_2 pressure. This is believed to be due to the return of previously sequestrated blood to the circulation as vascular constriction is relieved. Whereas a certain rise in PCO_2 after bicarbonate infusion can be expected, there is also a rise in the haematocrit that occurs at the same time; and by the swing in the capillary blood from the venous to the arterial side as shown on the Siggaard-Andersen Curve Nomogram. Figure 29 and 30 show a rise of PCO_2 and a drop of PO_2 after infusion (also Fig 31 and 32). These results all show firstly, a stagnant circulation with capillary stasis after capture changing to a normal peripheral circulation with arterialised capillary blood soon after infusion.

† Dr E. Young, Division of Nature Conservation, Pretoria.

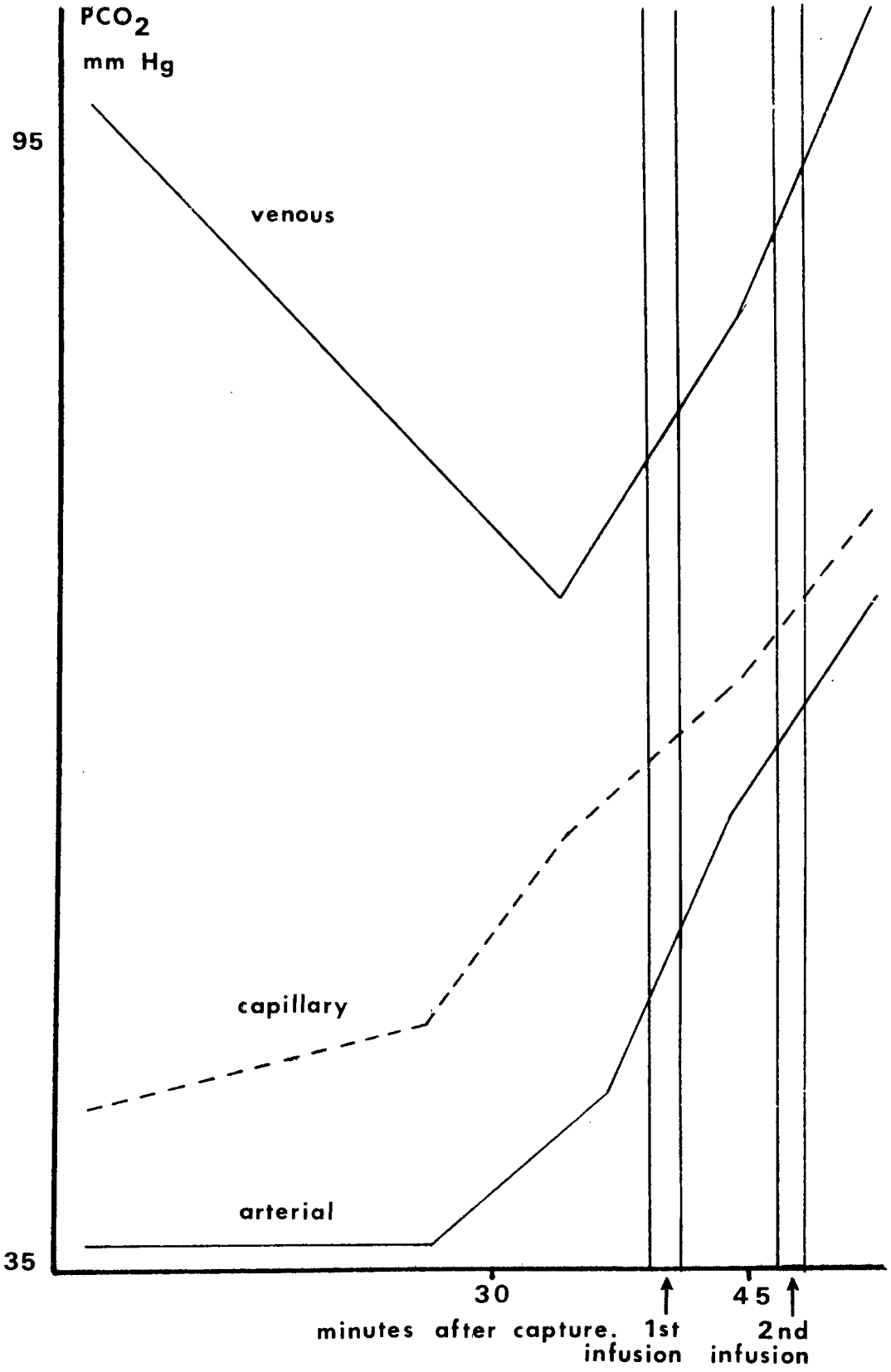


Figure 29: PCO_2 changes in blue wildebeest blood after capture and after bicarbonate infusion therapy.

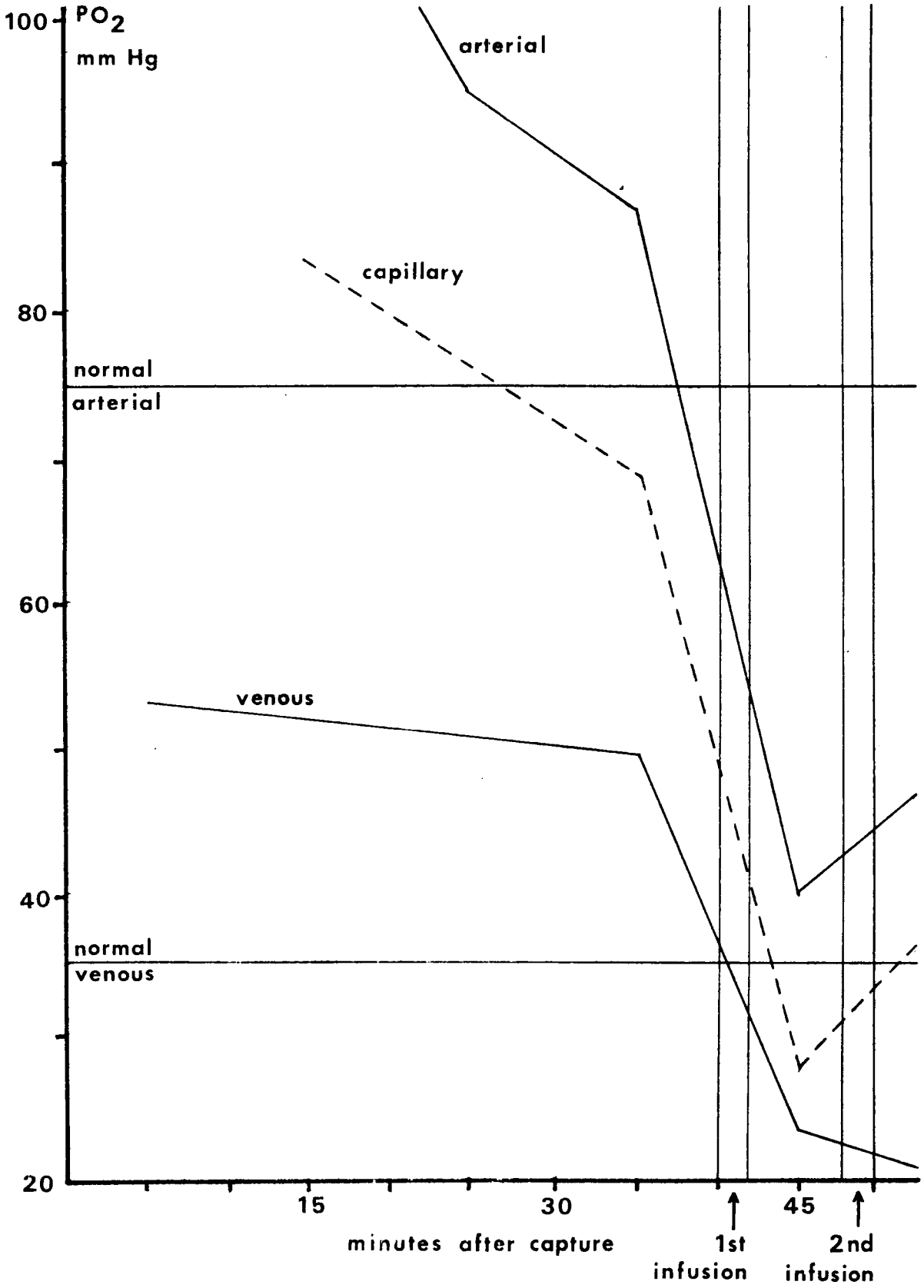
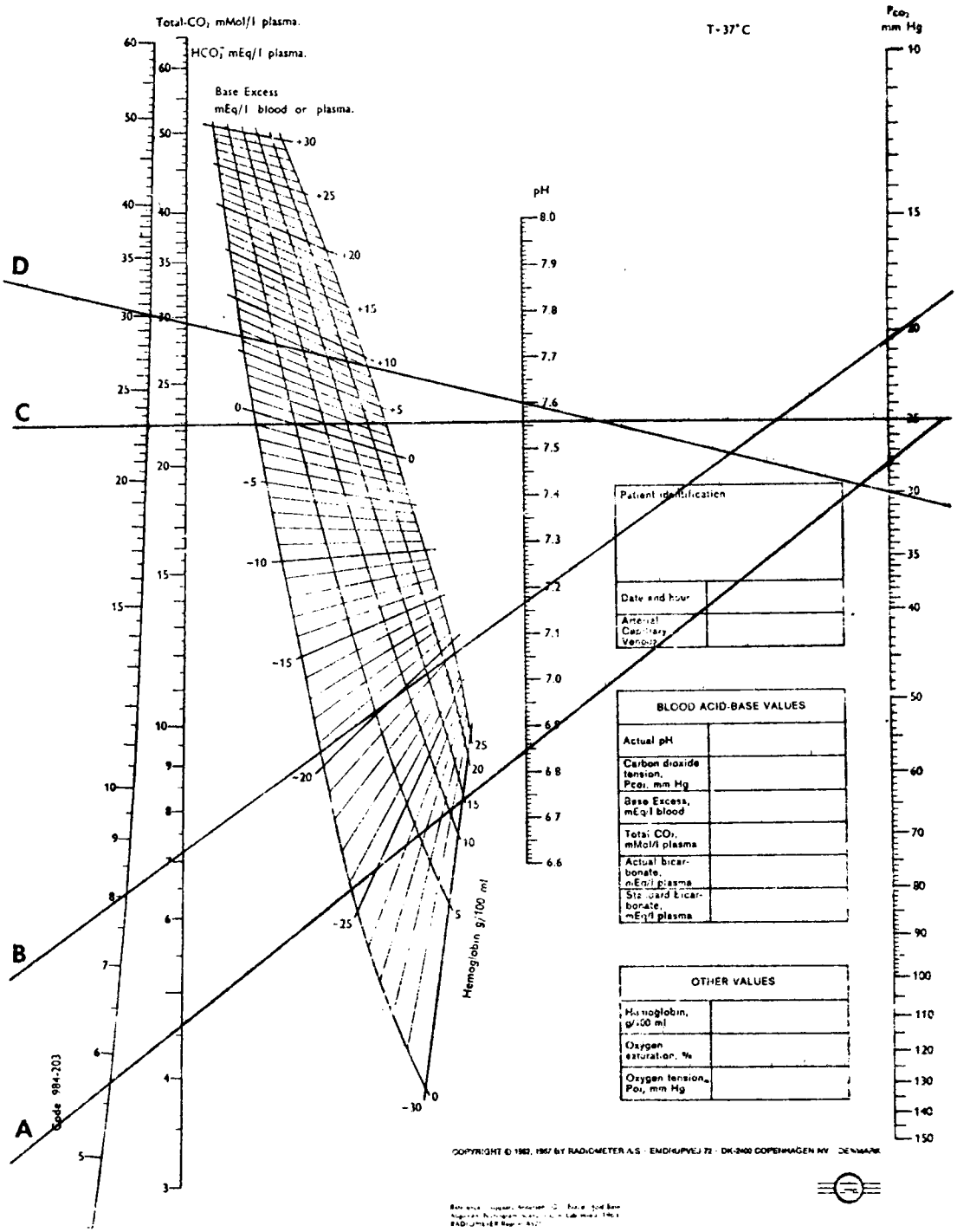
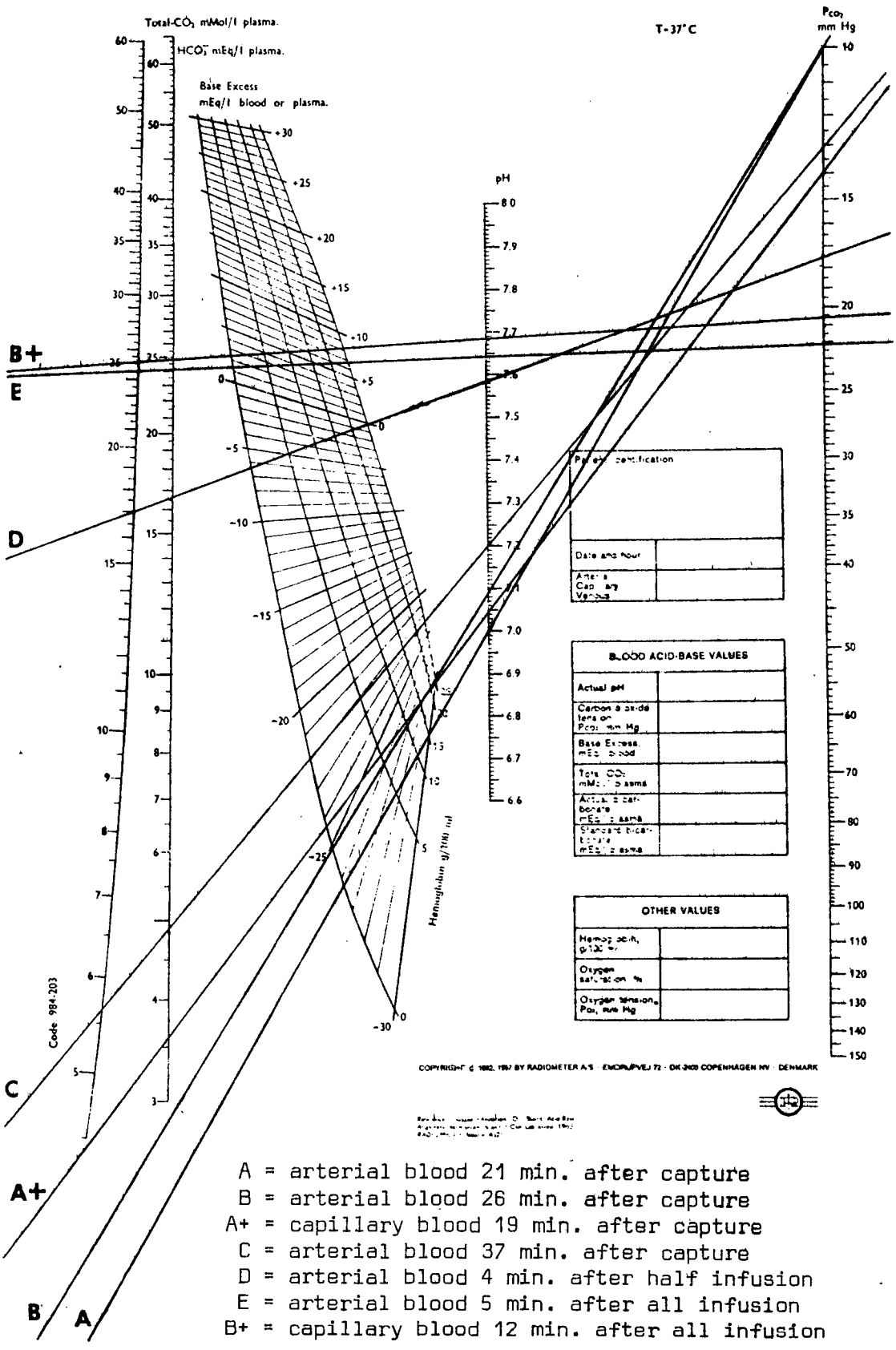


Figure 30: PO_2 changes in blue wildebeest blood after capture and after bicarbonate infusion therapy.



A = 7 min. after capture
B = 35 min. after capture
C = 4 min. after half infusion
D = 5 min. after all infusion

Figure 31: The Siggaard-Andersen alignment nomogram showing changes in mixed venous blood in blue wildebeest after capture and after bicarbonate infusion therapy.



A = arterial blood 21 min. after capture
B = arterial blood 26 min. after capture
A+ = capillary blood 19 min. after capture
C = arterial blood 37 min. after capture
D = arterial blood 4 min. after half infusion
E = arterial blood 5 min. after all infusion
B+ = capillary blood 12 min. after all infusion

Figure 32: Changes in arterial and capillary blood in blue wildebeest after capture and after bicarbonate infusion therapy.

The sharp drop in PO_2 levels seen in zebra, especially after infusion, occurs in the wildebeest between capture and infusion and is continued after infusion.

One possible contributing cause has been that of bloat. All our ruminants in 1974 tended to suffer from bloat which commenced immediately after capture. A possible cause has been the excessive rains in the Kruger National Park and the lushness of the grass during 1974 (see Chapter One). Also the increased availability of water ensured full hydration of the ruminal contents at all times of capture, the run tending to induce a froth in the ruminal contents, probably also exacerbated by reduced salivation and consequent increased ruminal acidity (Blood and Henderson 1974). The bloat necessitated repeated manoeuvres to relieve stomach gases and on several occasions near asphyxia occurred. On the other hand, PO_2 levels rose after the low post infusion level, and therefore this can be fairly ascribed to the same cause as those producing low PO_2 levels in the zebra.

The haematocrit level showed a rise at the same time as the PO_2 levels fell. This is a strong indication of a return of red blood cells to the circulation after having been cut off from the normal circulating blood by vasoconstriction and blood stagnation.

The marked difference in arterialised capillary blood PO_2 before and after infusion also indicated that the peripheral circulation was stagnant and cut off from the main

circulation, during the post capture period and that this condition was relieved by infusion therapy (Fig. 30).

The continued low level of blood pH until the time of infusion was not exhibited by any of the wildebeest. This suggests that recovery would have occurred unaided, although this is not necessarily true, as this trend is shown by some of the zebra, which died when untreated, i.e. the recovery is not rapid enough to prevent circulatory damage and shock. Capillary PO_2 levels show a continuous drop until the time of infusion after which there is a rise, further strengthening the belief that there is a deterioration of the peripheral circulation until that time. The PO_2 level of the venous blood falls after infusion, presumably due to the release of the capillary blood. The pH levels show a similar pattern except that the values (capillary and arterial) were close at the time of capture, diverged, and only closed again after the second half of the infusion (Fig. 33).

The pH level was somewhat higher in 1974 than in similar experiments performed in 1973 presumably as a result of the taller grass and general wet conditions slowing the actual chase, although this was not apparent from the actual speed and distance records. However, the small respites gained by the animal as the truck overshoots and has to circle to take up the chase may be considerable, while the vehicle does not actually either slow or show reduced mileage.

Correlation co-efficients and non-linear regression curves

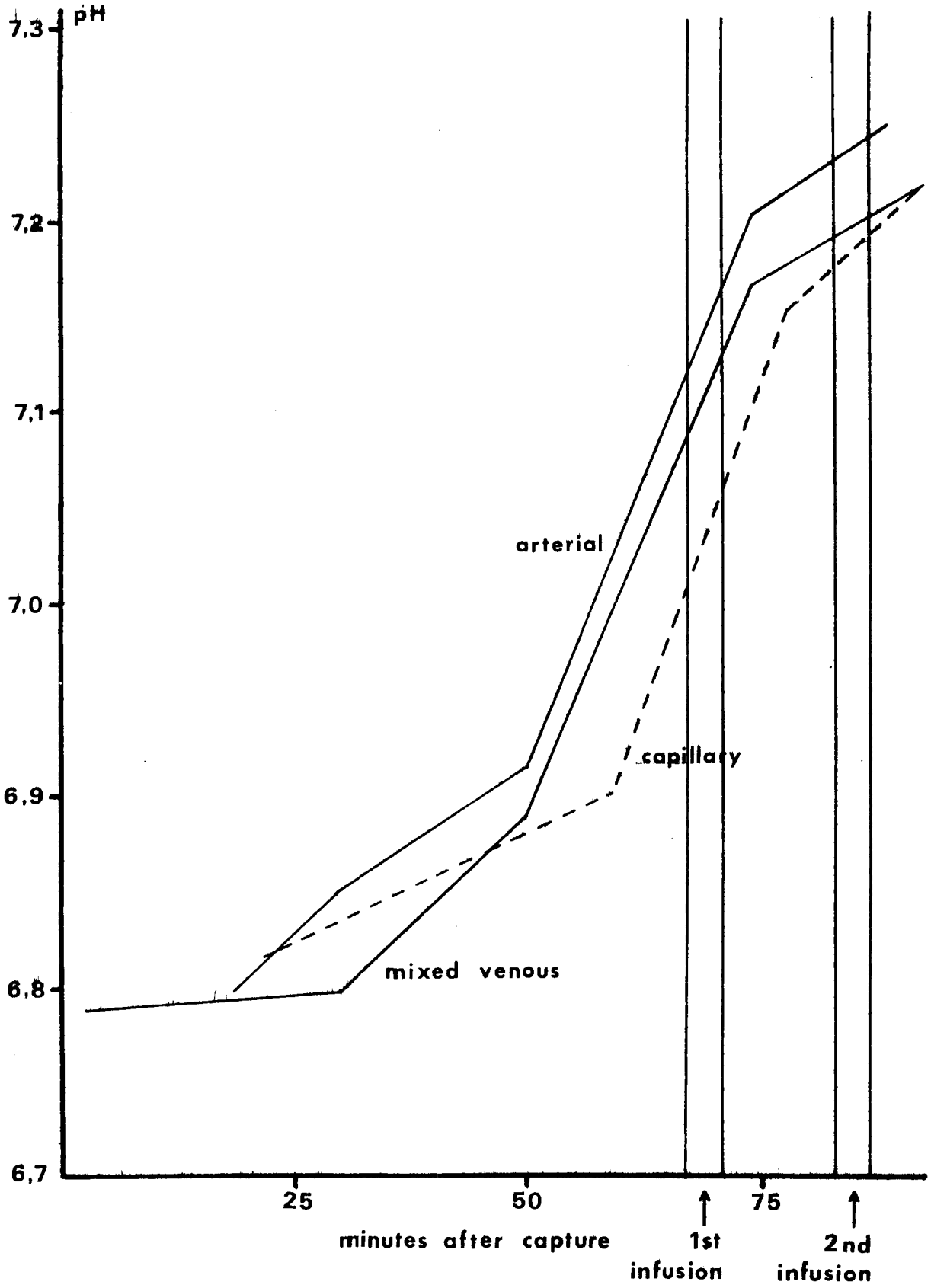


Figure 33: pH levels in zebra after capture and after bicarbonate infusion therapy.

were fitted to the data obtained before infusion and projected to beyond infusion times. The correlation coefficients are as follows:

zebra : $r_{xy} = 0,61078^{***}$ $(n = 27)$

wildebeest : $r_{xy} = 0,6683^{***}$ $(n = 14)$

The regression curve equations are as follows (Fig. 34 and 35):

zebra : $y' = 6,8593\ (1,000401)^{x}$

wildebeest : $y' = 6,966\ (1,00101)^{x}$

MUSCLE PH

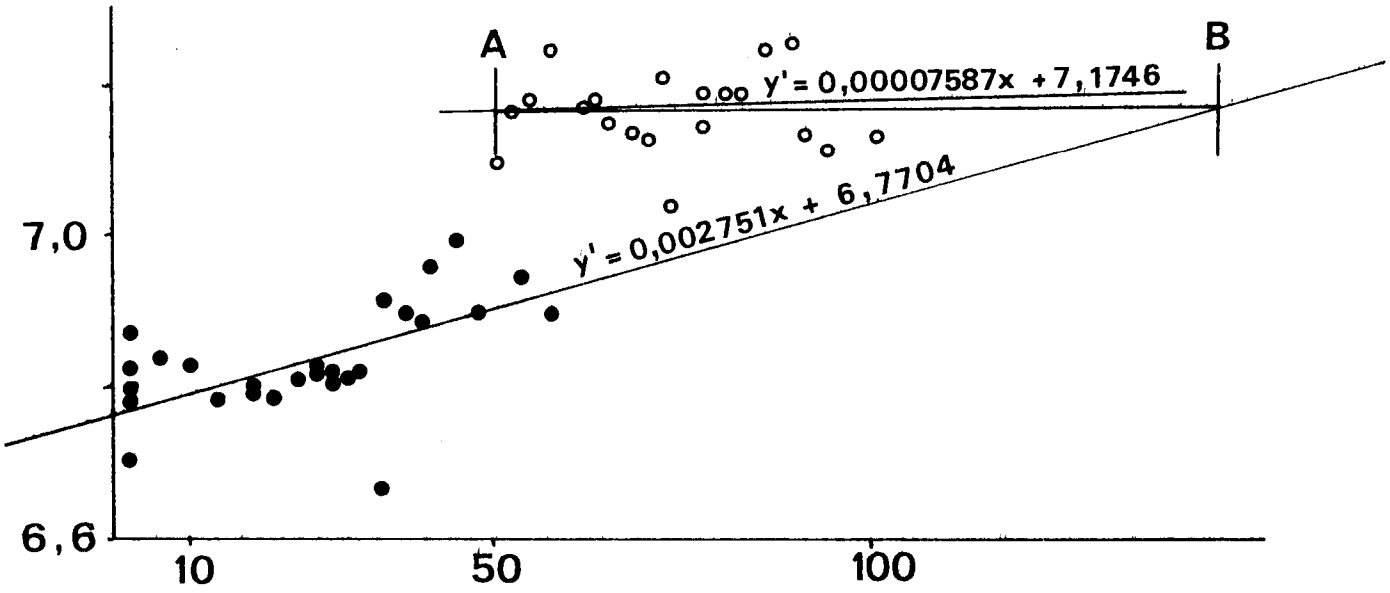
THE results showed variations between the pH of muscle and blood, which at one time were not thought to be of major significance. Subsequent investigation suggests, however, that the areas exhibiting a pH lower than that of the blood, were subject to a loss of normal tissue perfusion.

Nine values of muscle pH were compared to nine corresponding values of venous pH (Table 8) on two wild zebra. The *t*-test for paired values was applied as being the most suitable. The result was as follows:

$t_{8} = 7,203^{***}$ $(n = 9)$

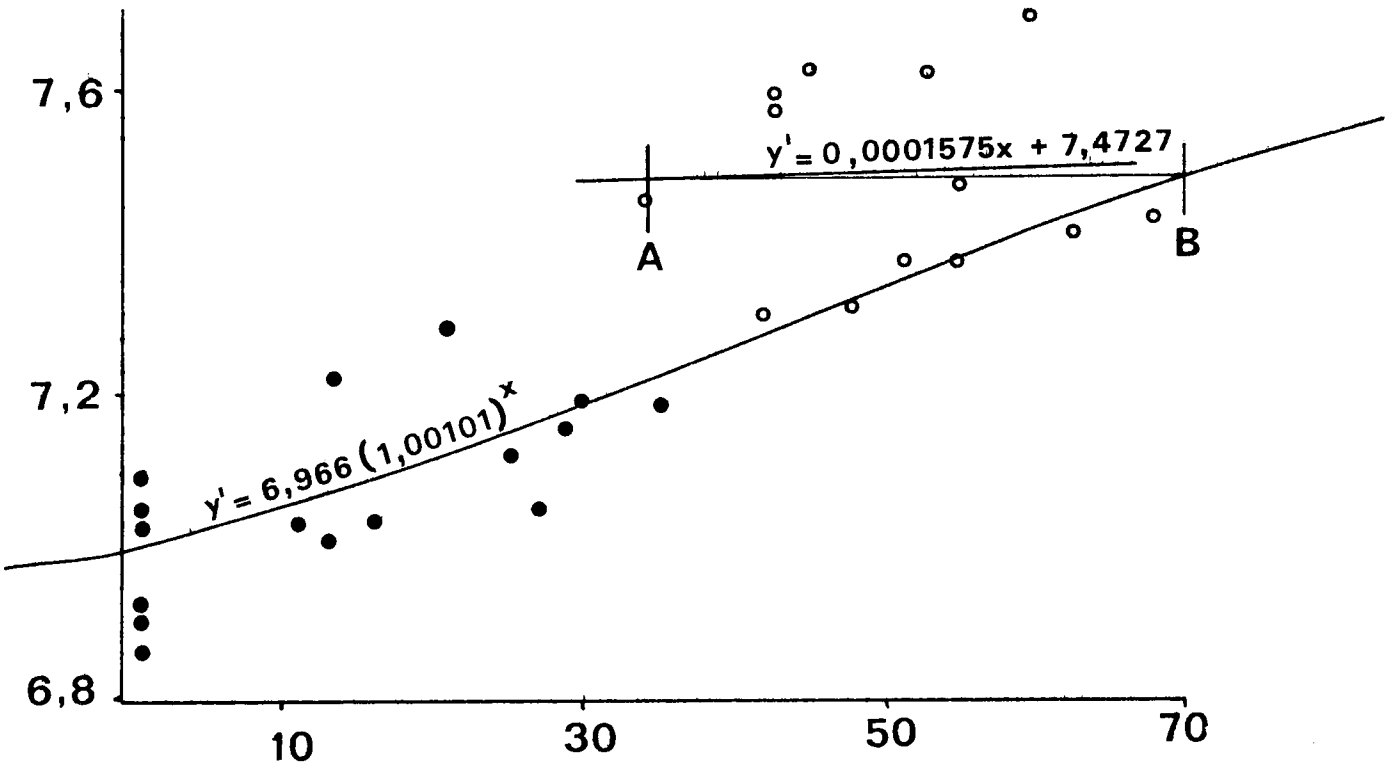
Muscle pH was *highly significantly* different from venous pH, and venous pH was also consistently higher than muscle pH.

● pre infusion
○ post infusion



min. after capture
Recovery time advanced 95 min. by infusion (A to B).

Figure 34: Regression curve of pH values before infusion and projected for post infusion values in zebra.



min. after capture
Recovery time advanced 36 minutes by infusion (A to B).

Figure 35: Regression curve of pH values before infusion and projected for post infusion values in blue wildebeest.

Table 8: Muscle pH compared with venous blood pH in zebra before and after bicarbonate infusion therapy after forced exercise.

	muscle pH	venous pH
zebra 1		
before release	7,15	7,04
after exercise	6,75	7,01
after 30 minutes	6,70	6,86
after 500 m-equiv. infusion	6,65	7,21
after 1 000 m-equiv. infusion	6,90	7,32
zebra 2		
before release	7,10	7,25
after exercise	7,00	6,94
after 500 m-equiv. infusion	7,00	7,22
after 1 000 m-equiv. infusion	7,20	7,39

DISCUSSION

BLESBOK

THE finding of moderately low pH readings in blood of blesbok chased and netted under simulated capture conditions, in itself came as no surprise. The extent of the role played by metabolic acid as compared to the respiratory component was, however, unexpected. Nearly all the blesbok

were normal with regard to blood oxygen (PO_2) and blood carbon dioxide (PCO_2) values, or else were actually hyperventilated, i.e. there was more oxygen, and less carbon dioxide present in the blood than when at rest. In spite of this the blood pH was down to below what is normally considered as tolerable levels (Tables 5 and 9).

Table 9: Blood pH in blesbok immediately after exercise.

group	no. in group	minimum	maximum	mean
all	18	7,07	7,45†	7,22
1	6	7,07	7,40	7,20
2	8	7,25	7,45	7,28
3	4	7,26	7,59	7,35

† discounts one exhausted animal in Group 3

Another factor that was contrary to expectation was that the animals that ran the shorter distance showed the more profound fall in blood pH. Also these same animals tended to exhibit higher pulse and respiratory rates (Table 10).

Although there is no statistically significant correlation between any of the acid-base parameters and distance run, the following deductions can be made: PO_2 lies within the 10 to 25 percent significance range; of the positively correlated parameters, pH values rose (i.e. animals became less acidotic) the further the distance run, the standard

Table 10: Pulse, respiratory rates and body temperature in blesbok immediately after exercise.

distances run (km)	pulse	respiration	temperature (°C)
2	250	64	41,3
4	128	96	41,6
8	228	84	41,8
8 - 10	112	40	41,6
10+	204	84	41,8

bicarbonate, base excess and PO_2 rose proportional to the distance run; the negative correlation with PCO_2 indicates that PCO_2 decreased as the distance was increased.

When the pH of the blood samples, after equilibration with 4 percent and 8 percent CO_2 was plotted against log CO_2 on a Siggaard-Andersen Curve Nomogram, we see the strongest shift in those animals than ran rapidly for 2 km. The animals in that group showed lower pH values than those that ran 4 km, while the only animal that became exhausted shows a line that lies to the right of normal at the time of sampling, moving slightly to the left of normal 10 min. later.

Although the group of 18 animals is too small in itself for valid conclusions under the circumstances of the experiment, the indications are that a rapid build-up of fixed acid occurs during the early part of strenuous exercise, and that as the

animals become progressively exhausted, the pH rises.

This general picture may explain why an unexpectedly high mortality was suffered when tsessebe were injected from a helicopter after a very short chase, with synthetic morphine immobilising compounds as reported by Young (1972). Morphine and morphine-like compounds are generally regarded as causing acidosis through reduction of the sensitivity of the respiratory centre (Soma 1971).

The effect of this may have been to cause a further reduction of the blood to levels incompatible with survival. On the other hand, tsessebe injected with etorphine hydrochloride/acetylpromazine maleate mixture while standing, exhibited no acidosis, the blood pH level remaining well within normal limits (Fig. 22). It may then be postulated that the intensity of the efforts made by the antelope to escape from the helicopter alone may have caused pH changes sufficient to cause death.

The extent of the acidosis manifested in this series of experiments is considerable by human standards although much lower values were later recorded for zebra and wildebeest. Already these levels are sufficient to affect myocardial contractility which is said to be depressed at pH levels below 7,15. In addition, according to Soma (1971), lower than normal pH is stated as creating a broad spectrum of homeostatic alterations including not only many metabolic and enzymatic activities, but also functional changes such as

cardiac contractility and rhythm.

The rise in levels of enzymes (GOT, GPT, CPK and LDH) indicates muscle and organ damage, stress and impending shock (Chapter Ten).

Zebra And Wildebeest

It appears that the immediate clinical manifestation of capture myopathy due to an acute chase is associated with a marked rise in hydrogen ion concentration of the blood. Bicarbonate and base levels in these animals together with blood PCO_2 indicated that the acidaemia was of metabolic rather than of respiratory origin.

The results in zebra, as well as those of antelope such as blesbok suggest that at least part of the capture myopathy syndrome may be ascribed to a profound fall in blood pH. The fall in pH to levels of 6,5 after only a short chase indicates that acute capture myopathy is likely to occur in well-rested animals in good condition. Furthermore, the rapid build-up of metabolic acid in zebra exercised intensively for a short distance suggests that the precept of a rapid short chase is not necessarily sound and that animals are perhaps more likely to survive after a longer but less intensive chase.

At the levels of acidosis found after chasing and capture the contractility of the heart muscle and therefore the

cardiac output is considerably decreased. Watts and Webb (1969) report that myocardial contractility is depressed at pH levels below 7,15. Severe acidosis is stated to occur at a pH of 6,86 by Zimmerman and Levine (1957) resulting in a fall of blood pressure and an irritable myocardium with possible ventricular fibrilation and cardiac arrest. Levels as low as 6,8 have recently been reported as occurring in human athletes under conditions of intensive exercise by Osnes and Hermansen (1972).

The picture is inevitably complicated by a number of factors including the actual method of eventual restraint, subsequent fear and shock. Some of these may in themselves precipitate a state of capture myopathy, and resultant death. Moreover, factors such as genetic or acquired predisposition cannot be excluded, neither conditions of a more chronic nature, such as damage to major organs by shock, trauma, or lesions that may cause disease and death after longer intervals following capture. Deaths in this group were, however, of the acute nature occurring in less than 12 hours in all untreated zebras, suggesting that in this animal species the pH factor described, alone militates against survival even though the actual chase was short and the stress inflicted relatively slight.

CAPILLARY BLOOD

CAPILLARY blood samples are taken routinely in hospitals to reflect the pH and gas pressures of arterial blood. A series of blood samples taken, as far as possible, simultaneously

from arterial, venous and capillary sources showed significant differences that appeared to relate to the state of circulatory stress. Arterialised capillary samples taken soon after capture when plotted on the Siggaard-Andersen Curve Nomogram closely follow the arterial alignment (Fig. 36). As the animals became more depressed and the blood pressure measurements indicated blood circulatory embarrassment, the capillary values shifted to the venous side and beyond (Fig. 37). The shift was reversed after infusion so that the capillary values began to re-approach that of the arterial value (Fig. 38).

The occasional capillary showing PO_2 values exceeding that of arterial blood indicates that there is a slight tendency for equilibration of the blood drop with the atmosphere to take place. This tendency is enhanced in our experimental animals in that a dependant drop is difficult to obtain so that blood runs down the ear forming an excessively large surface for gaseous exchange to take place. The variation of the capillary parameters between that of the venous and arterial sides appeared to parallel the degree of stress and circulatory restriction suffered by the subject animal.

Differences in the parameters of capillary bloods as compared to those of arterial blood occurring in stressed animals indicate that the capillary method could not be used as a substitute for arterial blood under these conditions.

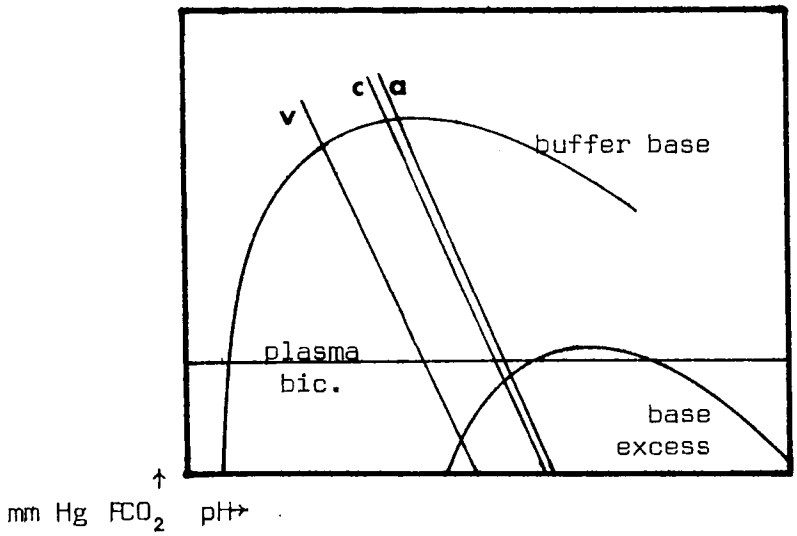


Figure 36: Capillary blood at rest. Siggaard-Andersen curve nomogram showing relationship with venous and arterial values.

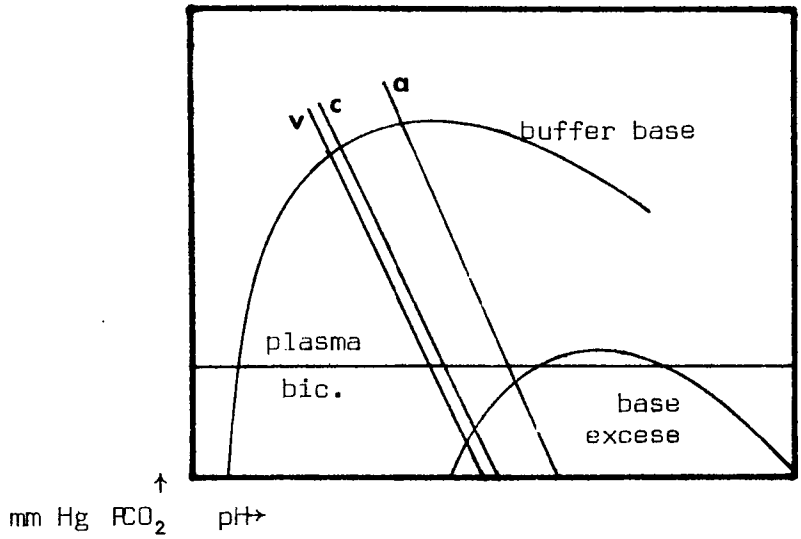


Figure 37: Capillary blood during stress. Siggaard-Andersen curve nomogram showing relationship with venous and arterial values.

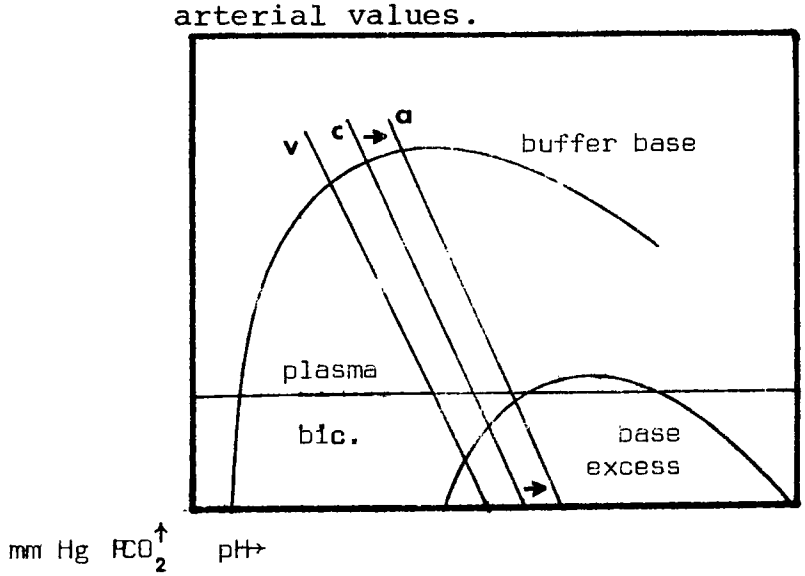


Figure 38: Capillary blood during recovery. Siggaard-Andersen curve nomogram showing relationship with venous and aterial values.

Recovery After Infusion

Along the regression curves of pre-infusion data were fitted the post infusion data. If a line were projected from the regression to reach the point that infusion data started at, we can see how much time is gained or not, by the infusion. This does not take into account other physiological damage occurring meanwhile.

There was a case, however, of one zebra in the data analysed, which showed a negative correlation. If the line were to be projected into the future, it would indicate that without infusion therapy (which restored the pH) the animal would not recover.

There is a difference in the regression curves between zebra and blue wildebeest, and it is interesting to note that the starting values of pH were higher in wildebeest than in zebra, and each projected a different curve (Fig. 34 and 35). One could hypothesise that different starting values would project different curves as seen in Fig. 39, but the amount of data is insufficient to prove statistically.

Muscle pH

Work has been performed (Filler, Das and Espinosa 1972) on neonatals (man) in which rapid haemodynamic and respiratory changes were anticipated, such as surgical cases of gastroschisis, hepatic lobectomy and repair of cardiac defects.

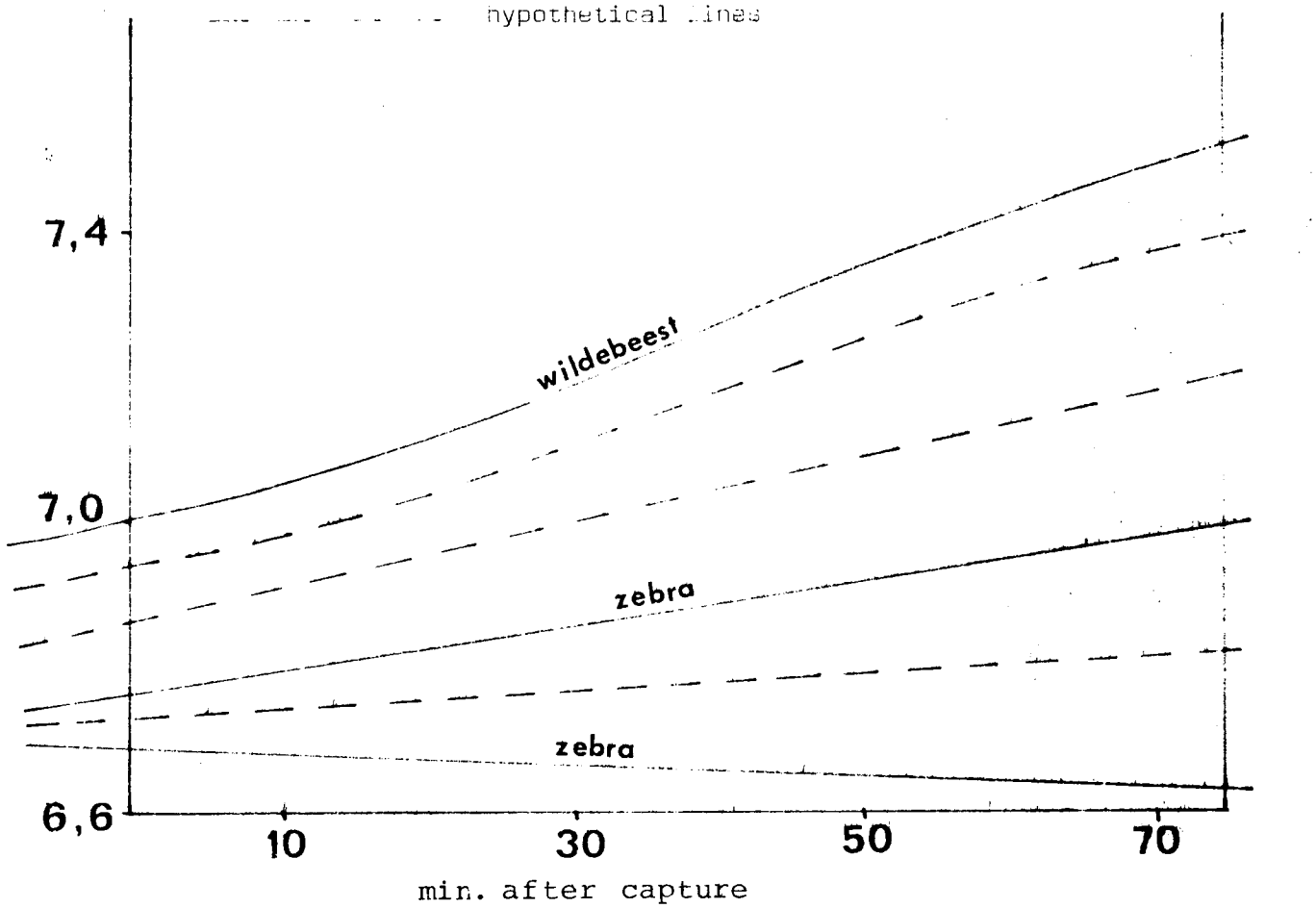


Figure 39: Hypothetical series of regression curves for various stages of starting values in pH levels projected over existing data.

Cases that died as a result of surgery showed a progressive *ante mortem* fall in muscle pH to values as low as 6,60 while the *blood pH value was normal or elevated*. The infusion of alkaline substances caused a rapid rise in blood pH to alkalotic levels, but failed to reverse a slow and downward trend of muscle pH. It was also observed that 'muscle pH as low as 7,08' lasting from four to 12 hours, and which did not improve with fluid therapy, were seen in cases due to severe anoxic brain damage. This condition was presumed to have resulted in a generalised vasculitis and persistent vasoconstriction.

In a number of straightforward cases of metabolic acidosis, the muscle pH reflected accurately the pH of blood. However, in other cases, such as those associated with renal insufficiency, muscle pH was 7,16 and blood pH 7,24. The muscle pH rose to that of the blood level after fluid therapy but only after a lapse of approximately six hours. It was concluded that a low muscle pH associated with normal or even high blood pH was due to abnormalities which are known to result in tissue perfusion such as bleeding, extra-cellular fluid deficit, decrease in cardiac output and the various conditions which cause peripheral vasoconstriction (Filler *et al* 1972).

Hypovolaemia due to the constriction of the major abdominal vein was reflected in a fall of muscle pH during a fifteen minute period and this fall of muscle pH preceded the fall in arterial blood pressure by five minutes, muscle pH returning

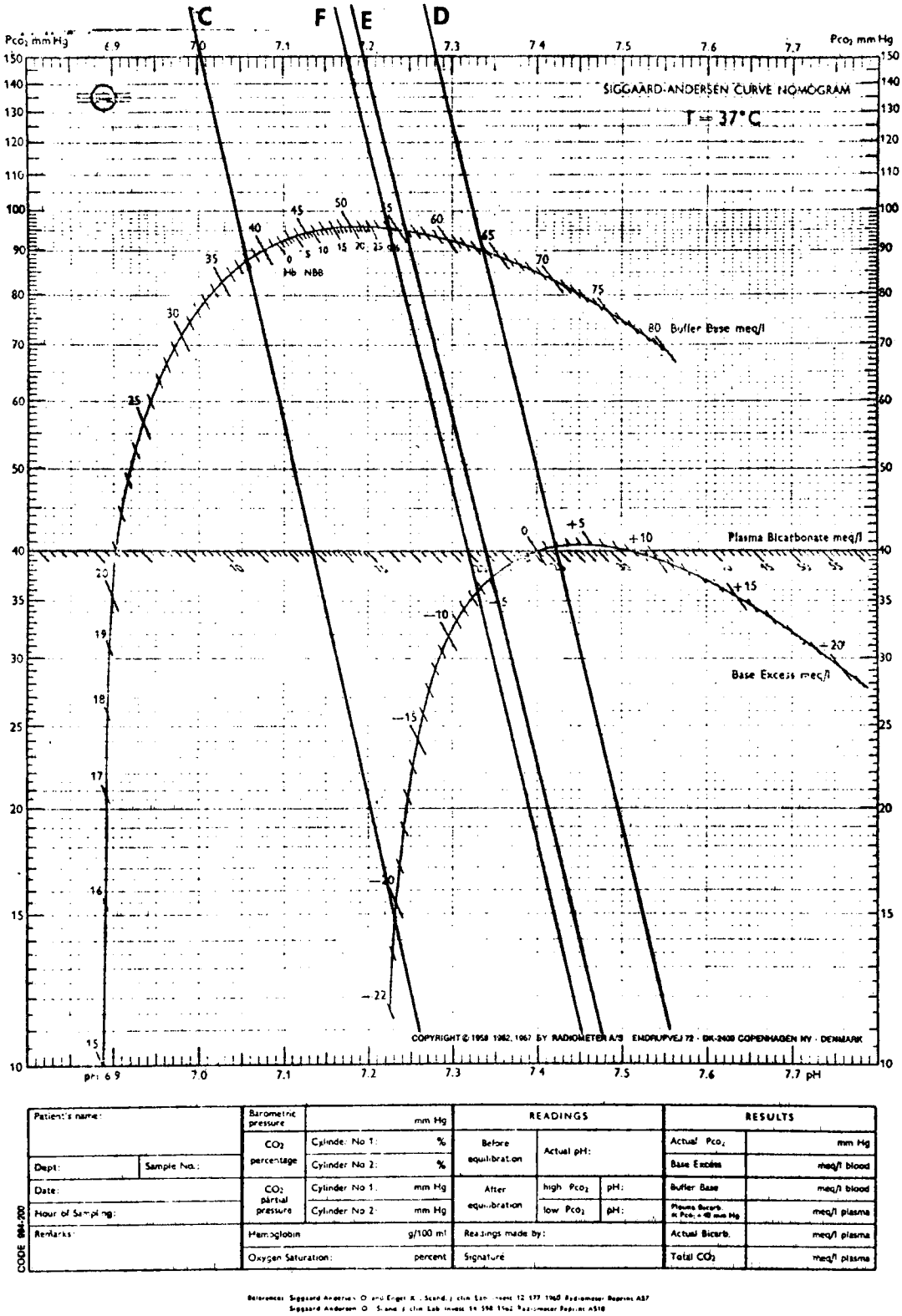
rapidly to normal when the circulation was re-established (Filler and Das 1971). It is significant to note that a *muscle pH lower than that of the arterial and central venous blood was seen right at the onset of shock.* Here again the blood pH gave little indication of that of the muscle. Animal studies by Smith, Lemieux and Couch (1969) also showed marked changes in muscle pH with minimal alterations in central arterial pH.

The measurements of muscle pH in zebra were made after the establishment of standard parameters and the drawing of the first series of blood samples, i.e. after catheterisation of the pulmonary artery and of peripheral arterial puncture. This procedure usually took ten minutes so that there was adequate time for the equilibration of muscle fibres with the circulating blood under normal conditions during rest. Ventilation during this period was adequate as indicated by optimum oxygenation of the blood and relactively low PCO_2 levels. It must therefore be concluded that the lower muscle pH value as compared to those of the circulation reflects significant changes in the normal circulation. A few measurements made so far corroborate these findings revealing distortion of the electrocardiogram and low systemic blood pressure (Chapters Five and Six). There is every indication, therefore that during the acidotic phase after capture there is a considerable fall in cardiac output with subsequent hypovolaemia.

It may be concluded that the animal stressed by a rapid

short chase and resultant acidaemia may exhibit a muscle pH that is lower than that indicated by the arterial and central venous blood. This appears to be due to a loss of essential tissue perfusion in turn resulting from a fall in cardiac output, reduced blood pressure and hypovolaemia. The lower muscle pH corroborates the findings of circulatory changes, e.g. low capillary PO_2 and high PCO_2, with high capillary haematocrit (Chapter Eight). Also this may in turn be responsible for the patchy discolouration of muscle fibres which is seen on autopsy of animals that die from the capture myopathy syndrome.

The restoration of the low muscle pH after infusion may be due directly to the bicarbonate in the perfusate, but probably to a greater extent due indirectly to the improved circulation. Filler *et al* (1972) found that the infusion of alkalis *did not affect muscle pH*, but then their patients were not suffering from impaired circulation as a result of acidosis but rather from an acidosis due to impaired circulation. The further fall in pH after restoration of normal blood levels with bicarbonate infusion does suggest a reservoir of metabolic acid in the muscles which is liberated during the hour or so subsequent to infusion and presumably only after the normal circulation and therefore tissue perfusion has been re-established (Fig. 40).



C: after 250 m-equiv.
D: after 500 m-equiv.
E: one hour post infusion.
F: two hours post infusion.

Figure 40: Siggaard-Andersen curve nomogram showing acid-base levels in zebra after bicarbonate infusion therapy.

CHAPTER FIVE

CARDIAC CHANGES

ELECTROCARDIOGRAM

INTRODUCTION

THE electrocardiogram is an important tool for the diagnosis of conditions affecting the heart, or those manifesting themselves in changes of the heart beat. It has, however, been used to a much lesser extent for animals than for man, and similarly for wild animals than for domestic stock. The literature on cardiac defects in animals measured in this way is sparse, other than a relatively small amount on the dog and cattle (Volkart 1957, Mordohovich 1971) and the horse (Steel 1963).

A further problem is associated with the instrument itself. The electrocardiogram in man is based upon the original work of Eindhoven, where the human body is considered as a flat triangle. The potential difference is taken between the left and right arms, the right arm and left leg, and the left leg and left arm. The relationship of the heart to the limbs in animals is clearly quite different. Considerable confusion has arisen in that it has only recently been realised that the electrocardiogram of the animal shows considerable differences in relationship to posture, and

that there are changes in the configuration according to whether the animal is lying on its left side or right side. Similarly, experimental recordings in dogs restrained on their backs, show different electrocardiogram tracings according to whether the heart falls slightly to the left or the right in accordance with small variations in the animal's position (own records, unpublished).

Consequently, all animals recorded here have been instrumented while lying on their right side. Included are animals that were restrained manually after standardised exercise on the track, and those immobilised and infused with low levels of adrenaline with and without phenoxybenzamine hydrochloride. Details of the registration and application of the electrocardiograph leads are described in Chapter Two. The eleven tracings shown here reflect a selection of nearly a kilometre of heat-sensitive electrocardiograph tracings.

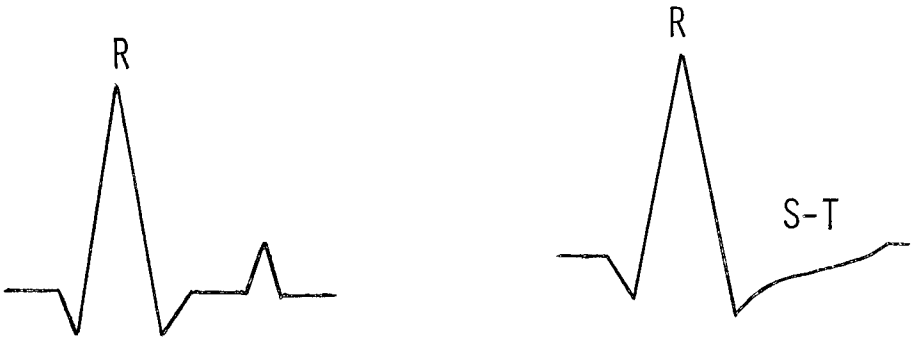
RESULTS

VARIOUS pathological complexes have been noted in the electrocardiogram tracings. These are the following:-

ISCHAEMIA

LACK of blood flow to part of the heart muscle causes this area to become negative when the heart muscle is stimulated. This occurrence, especially in the left ventricle, and consequently the abolition of part of the positive wave will

induce an inverted T-wave (Schamroth 1975), sometimes together with a raised ST-segment. This configuration, however, depends upon the lead from which the particular part of the tracing is taken and its relationship to the ischaemic area. In animals such as the horse the normal T-wave tends to be inverted (Steel 1963). Ischaemia may be evident only on exercise when due to enlargement of the heart muscle. Then a normal tracing at rest will show changes immediately on exercise.



normal pathological

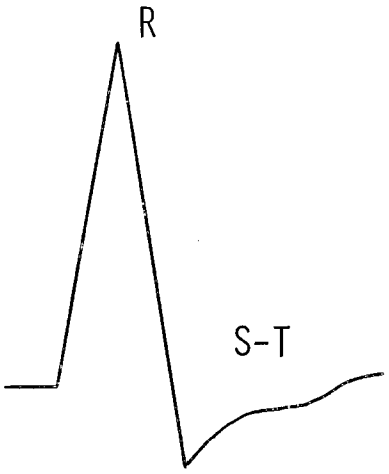
This phenomenon thus may occur in apparently normal hearts where there is excessive stress, and appears to occur in some of the tracings of the eland.

HYPERTROPHY

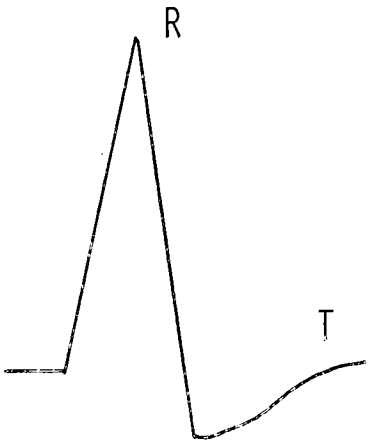
THE musculature of the heart tends to dilate excessively or thicken under certain conditions and has been noted in the zebra at necropsy. This may be due to pulmonary hypertension with regard to the right ventricle, and in the left ventricle in relationship to excessive work load. The *post mortem* picture has not been related to the electrocardiogram tracing, but indications of abnormality are evident.

Thus, if the right ventricle is enlarged, the potential may equal or exceed that of the left ventricle resulting in a greatly increased R-wave. At the same time, ischaemia will induce, in this case, a depressed ST-segment. When the musculature enlarges, there is a relative paucity of blood vessels, as the ratio of blood vessels to muscle fibres remains the same.

When the left ventricle is enlarged there is an increased electrical force in the left ventricular wall. Here again we have a high R-wave and the T-wave is inverted. This also appears in the eland after repeated forced exercise.



depressed ST-segment



high R- and inverted T-wave

Sinus Tachycardia And Bradycardia

These two conditions are not necessarily associated with disease conditions. The first appears to occur in the test animals under conditions of hyperthermia or excitement. Heart rates rising to 200 or more per minute have been recorded especially in zebra. The configuration of the electrocardiogram is essentially normal but rapid, and changes

that occur are probably due to incomplete diastolic filling. Bradycardia on the other hand is associated with vagal hypertonus. It tends to occur irregularly, and is often associated with periodic vagus escape. It was seen only in the eland bulls.

Sino-auricular And Atrio-ventricular Block

This is due to a periodic failure of the conductive system, and one or more complete configuration fails to materialise (Schamroth 1975). It may occur regularly on alternate beats, or sporadically. If it occurs on alternate beats it may be confused with bradycardia, but in this condition the heart rate will double suddenly after a relatively small effort. Partial block with lengthening of the PR-interval is evident. It is likely that this phenomenon in wild animals is associated with cardiac damage from adrenaline effects during restraint and thus more likely to be more damaging to the heart than it is on an animal free to move. In partial block, the PR-interval lengthens until sporadically a beat is dropped (see eland in Plate 10).

Ectopic Arrhthymias

These are ascribed to beats coming from the ventricular or other cardiac tissue. A beat arising in the ventricle cannot affect the atria, but the subsequent normal beat spreading downwards from the atria, may find the ventricles refractory (lower tracing, sable bull Plate 4), so that a pause in the normal sequence ensues with loss of a QRS-complex. Similarly, the QRS-complex may be deformed, particularly as

the impulse spreads through the muscle and not through the normal cardiac conducting fibres. This is seen in the tracings of the sable bulls (upper tracing, sable bull, Plate 4).

Paroxysmal Tachycardia

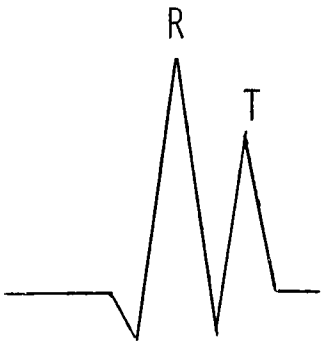
This is a series of rapidly occurring ectopic beats characteristically seen in Plate 1. The origin of the extra beats may be in the atria, the sino-auricular node or the ventricles.

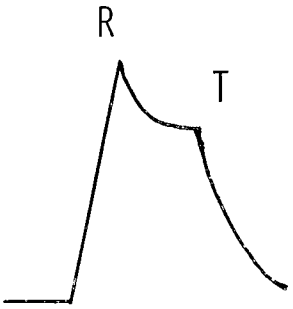
The extra beats seen in the animals in the present study were characteristically of high voltage and stand up well above the pattern of the other QRS-complexes. They have been observed mainly in animals with high catecholamine tonus and appear to be due to a raised irritability of the myocardium (see the high voltage complexes in tracing from sable bull, Plate 1).

Potassium Effects Or Hyperkalaemia (see also Chapter Thirteen)

The results of excess potassium in the plasma usually associated with red discolouration from intravascular haemolysis or circulating myoglobin results in tall T-waves (see eland Plate 8) usually with a diminution of the amplitude of the R-waves. This may result in a blending of the RS-complex with the T-wave (see eland in Plate 3).

An apparently enlarged U-wave is seen in hypokalaemia.

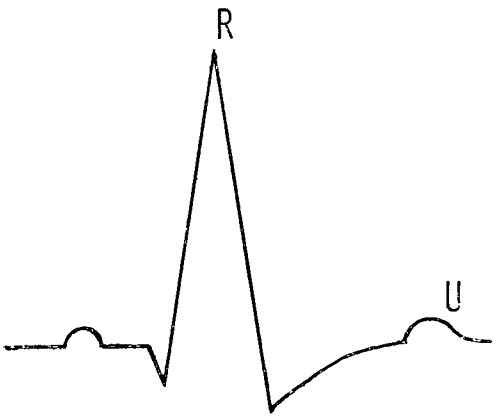




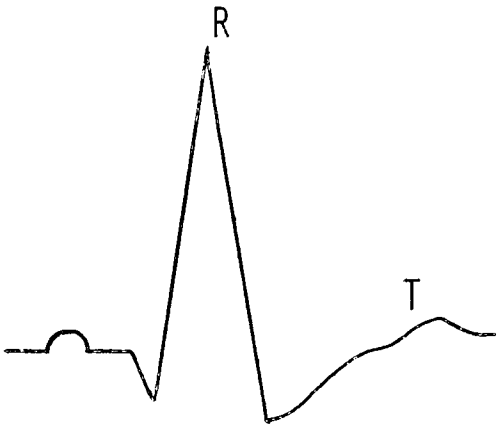
This condition seen in debilitated animals, animals with diarrhoea and with chronic intestinal parasitism, may be confused with hyperkalaemia while the U-wave may be confused with an enlarged T-wave. No definitive recordings of these have been made.

Calcium Effect (see also Chapter Thirteen)

In hypocalcaemia, due to depression of calcium ions by lactate, there is a slurring of the configuration and a lengthening of the QT-complex (see eland bull in Plate 7).



hypokalaemia



hypocalcaemia

The effect on the heart of lack of calcium ions is physiologically essentially the same as that of excess potassium.

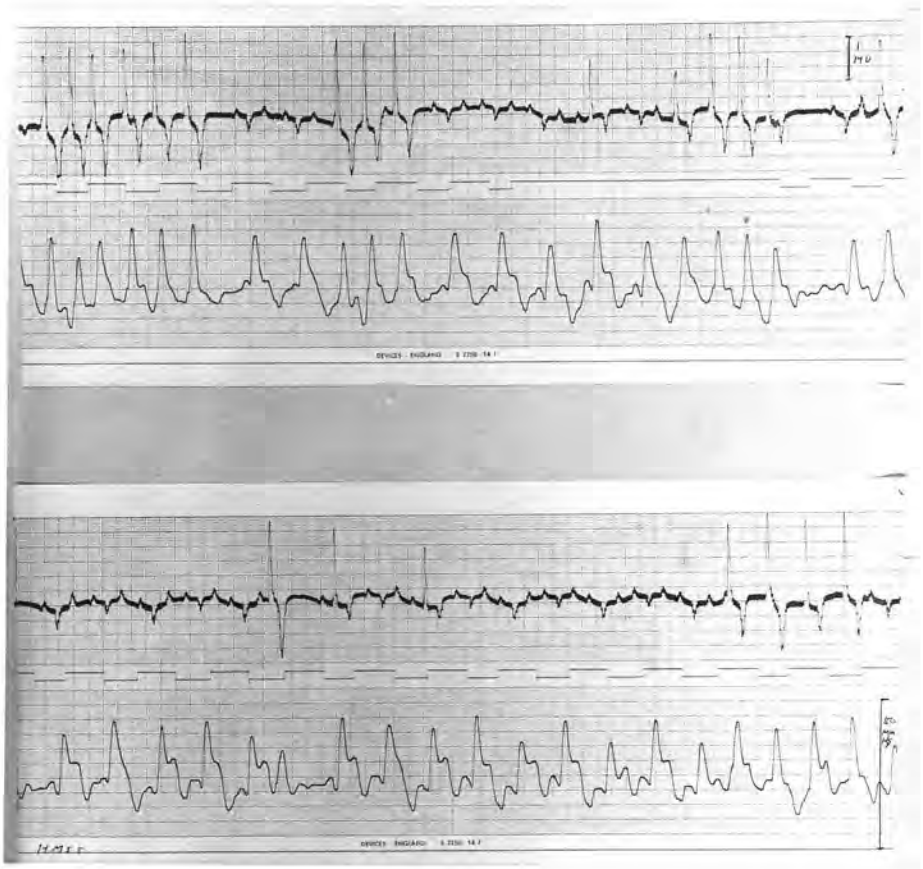


Plate 1: Electrocardiogram of sable bull under fentanyl/azaperone/xylazine narcosis. Infusion of adrenaline to examine blood urea, blood urea nitrogen, creatinine and lactate levels. The electrocardiogram shows systolic arrythmias with extrasystoles tending to occur in a trigeminal rhythm. The extrasystoles are reflected in changes in the pulsation of the pulmonary artery blood pressure. Towards the end of the upper strip a series of extrasystoles is followed by asystole. Pulmonary artery pressure is high. Extrasystoles are sometimes seen to be preceeded by a short coupling interval. Rapid ventricular tachyarrhythmias such as extrasystolic ventricular tachycardia, frequently preceed and predispose to ventricular fibrilation, which has been postulated as a cause of death in acute capture myopathy (probably near fibrilation is occurring in the right upper tracing).

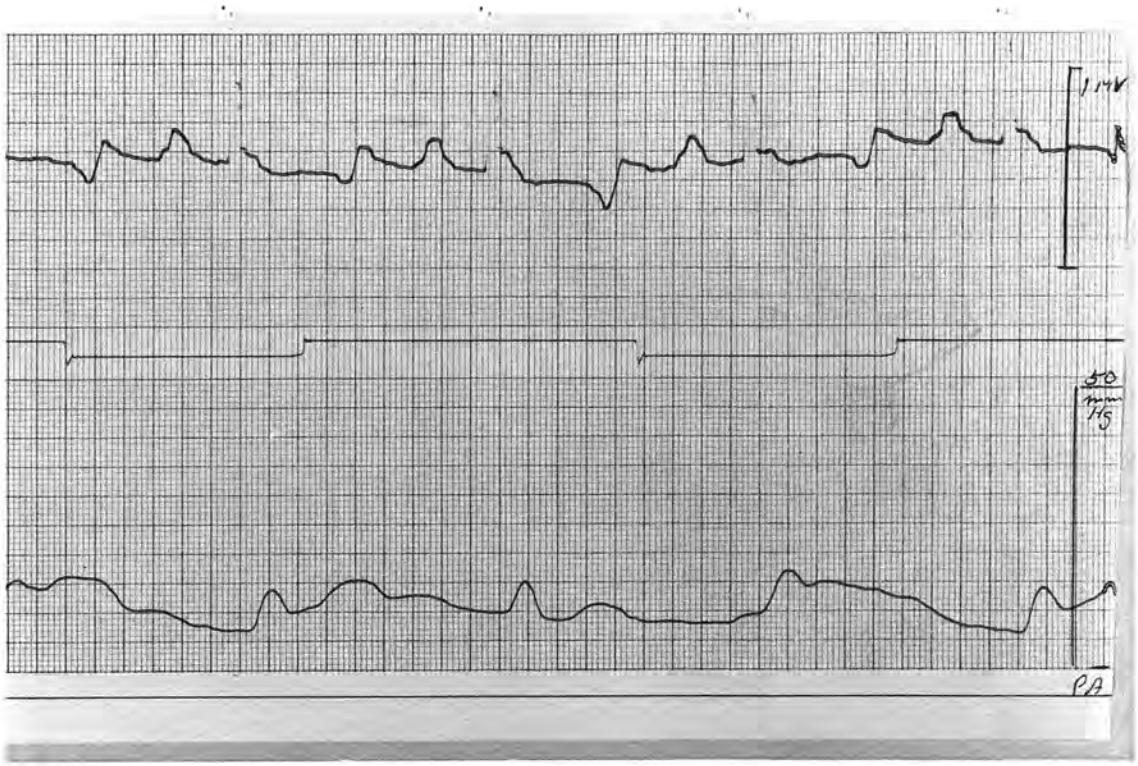


Plate 2: Electrocardiogram of eland bull 15 minutes after forced exercise over 2 km distance. There is a tachycardia with signs of atypical intraventricular conduction. This is reflected by the deformed and widened QRS-complexes. The blood pressure in the pulmonary artery is low with irregular curve configurations. Systemic pulse pressure in this animal was also low. The electrocardiogram configurations of this animal and the heart rate as well as the pulmonary artery blood pressure contrast markedly with the eland bull shown in Plate 3.

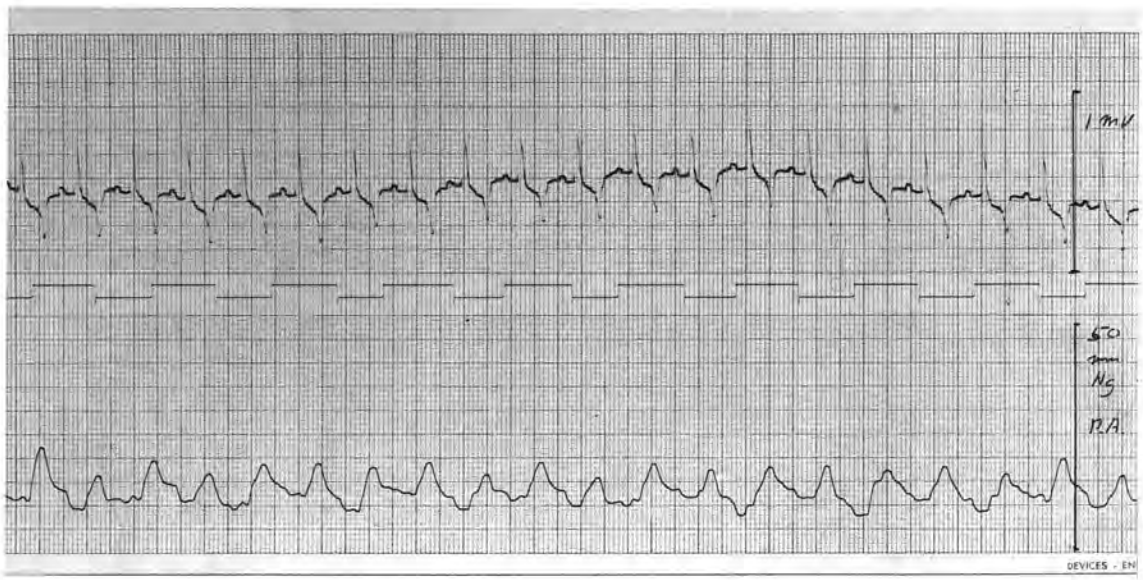


Plate 3: Electrocardiogram of eland bull 15 minutes after forced exercise over 2 km distance. The heart rate in this animal is more rapid with signs of ventricular tachycardia with changes in the QRS-complexes. P-waves are normal but T-waves are inverted and sometimes prolonged. The changes in the T-wave may result from a high level of circulating blood potassium. We have here, therefore, a rapid rate, arrhythmic sequence and secondary ST-segment and T-wave changes.

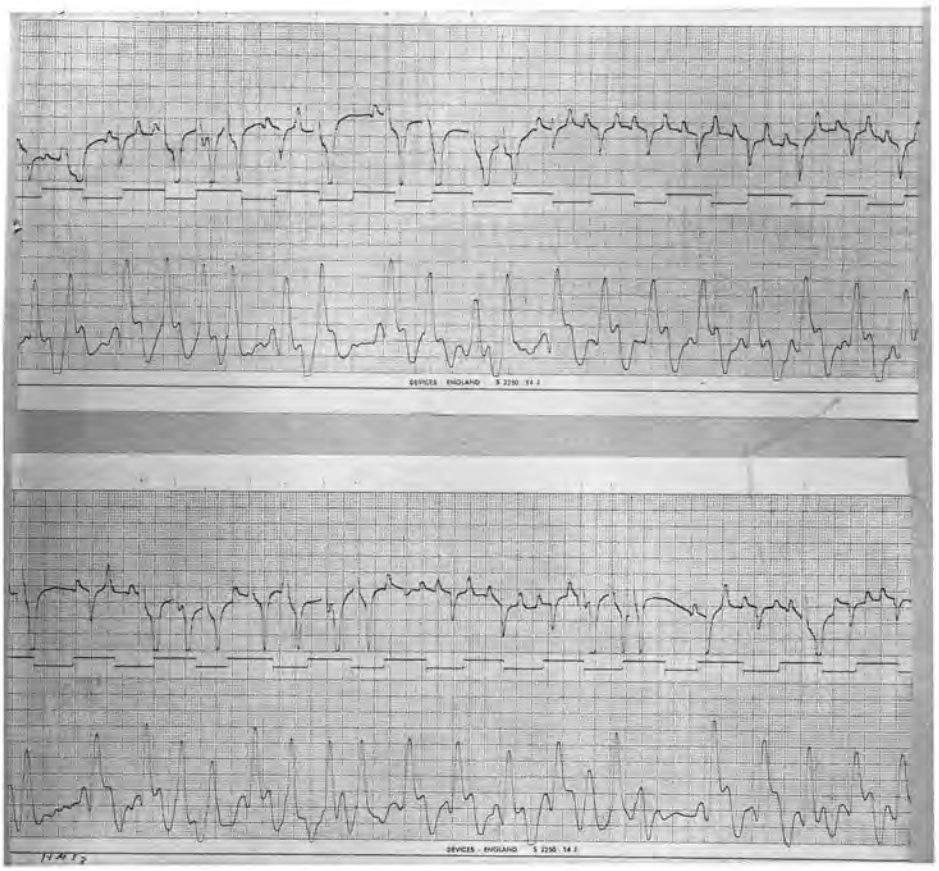


Plate 4: Electrocardiogram of sable bull with adrenaline infusion for urea, blood urea nitrogen and lactate estimations. Both tracings show paroxysms of repetitive extrasystolic ventricular tachycardia. The first bizarre complex of every paroxysm has a fixed coupling interval to the preceeding sinus beat. Some of the post-extrasystolic pauses are terminated by a P-wave different in configuration from that of the normal sinus P-wave (lower tracing). This probably indicates an atrial escape beat arising from an ectopic atrial focus.

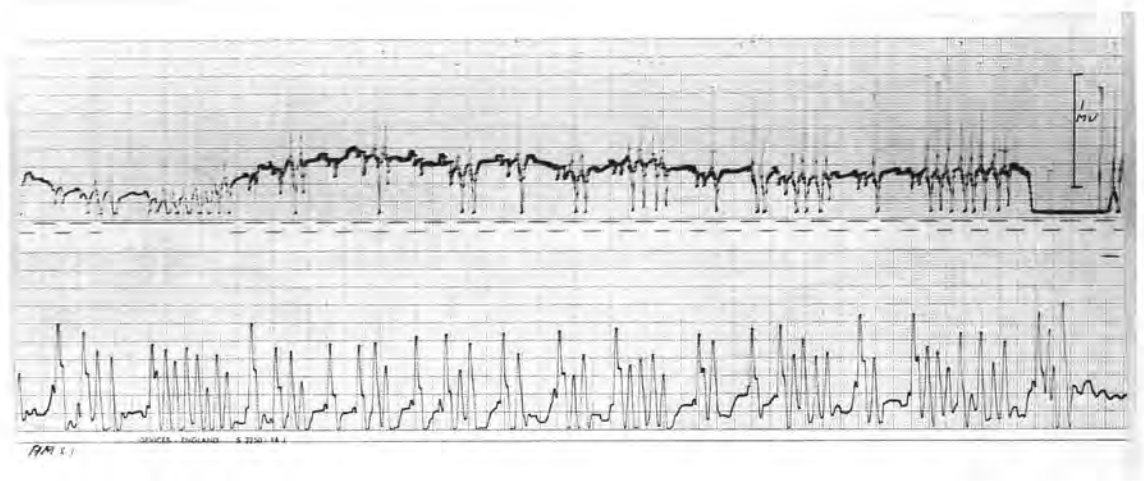


Plate 5: Electrocardiogram of sable bull with adrenaline infusion for urea, blood urea nitrogen, creatinine and lactate estimation. Seen in this electrocardiogram tracing are signs of atrial flutter. This is characterised by rapid heart rate and also by rapidly repetitive atrial deflection. These waves distort the base line of the electrocardiogram recording. Some of these atrial waves are superimposed upon and masked by the QRS-complexes, the ST-segments or the T-waves. Towards the end of the tracing there are signs of asystole or cardiac fibrilation with absence of ventricular pulsations seen in the tracing below.

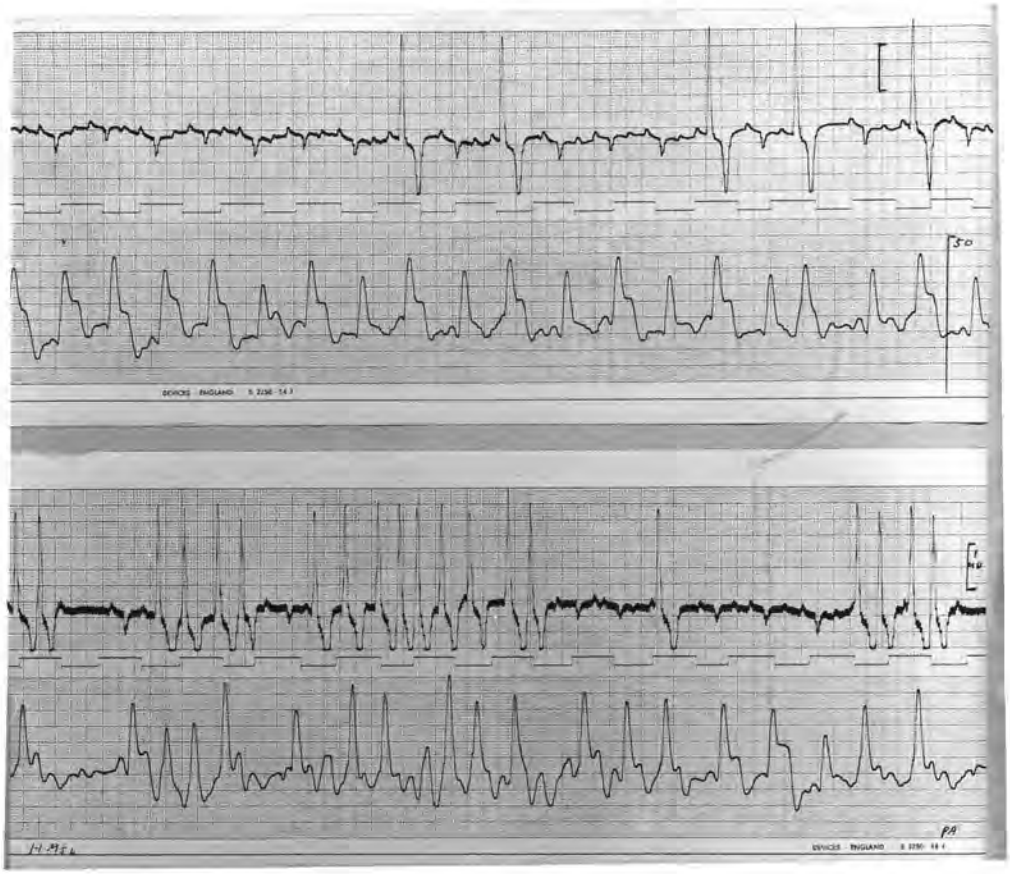


Plate 6: Electrocardiogram of sable bull with adrenaline infusion for urea, blood urea nitrogen, creatinine and lactate estimations. In contrast to the previous sable we notice here marked signs of ventricular extrasystoles, and high voltage extrasystolic ventricular tachycardia. We notice here the normal P-waves and ventricular extrasystoles in bigeminal rhythm (top tracing) and a longer series of consecutive ventricular extrasystoles in the lower strip. These lead to complex interference - dissociation. The extrasystoles lead, in this case, to high pulse pressure peaks in the pulmonary artery tracing seen particularly in the lower strip. In between the extrasystoles is a low voltage electrocardiogram (see 1 millivolt standard on lower tracing). In the second strip, the interval between the extrasystoles shows a low pulse pressure in the pulmonary artery tracing (see commencement of lower strip). Apart from that, the pulmonary artery pressure is fairly high, nearing the 50 mm mark, which probably represents adrenergic hypertonus and pulmonary vascular constriction.

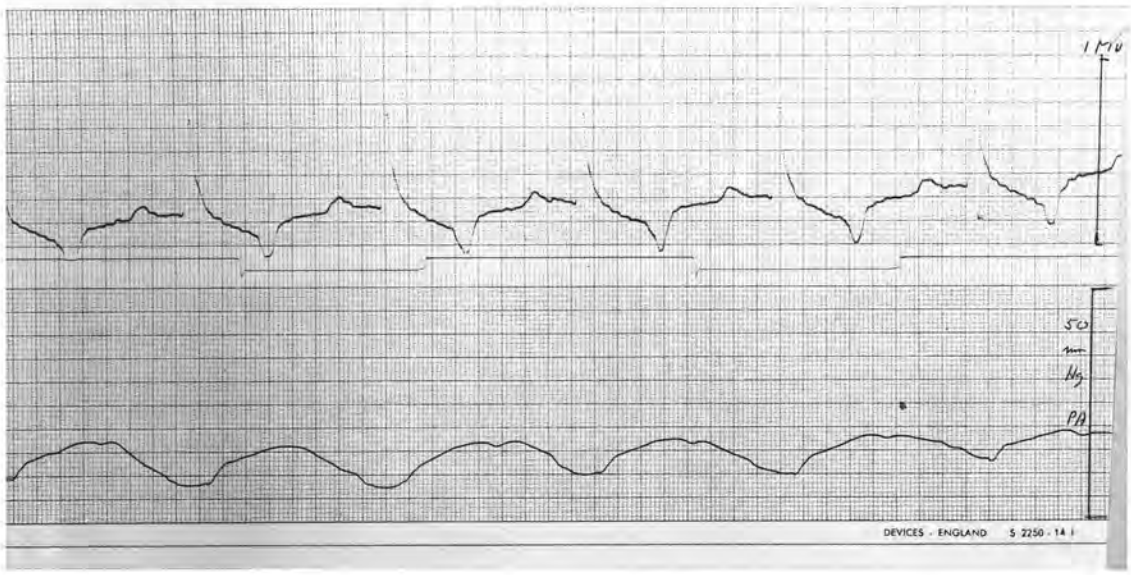


Plate 7: Electrocardiogram of eland bull 30 minutes after exercise over 2 km distance. We see here a slurring of the QRS-complexes and particularly the prolongation of the QT-interval has a typical hypocalcaemic effect due to the depression of calcium ions by lactate. The depression of the heart beat results in a low pulmonary artery pressure with marked absence of pulse peaks.

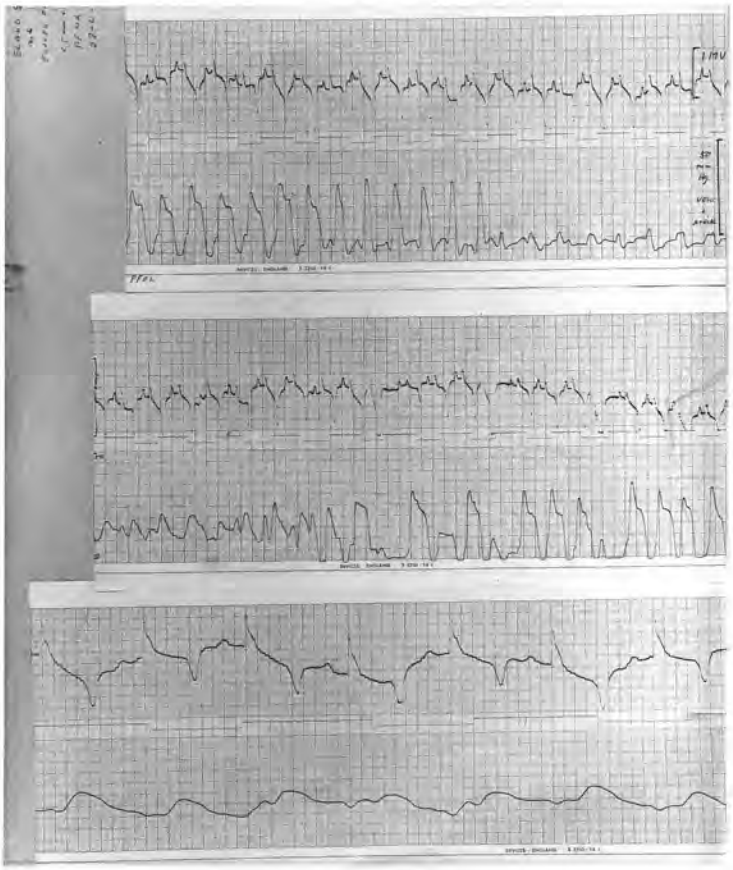


Plate 8: Electrocardiogram of eland bull, 45 minutes after forced exercise over 2 km distance. As in the preceeding plate, we notice a typical slurring of the QRS-complexes and particularly the prolongation of the ST-segment (see lower tracing). Seen also are high T-waves, possibly associated with a hyperkalaemia (see especially middle tracing). Ventricular pulse pressure waves are of normal amplitude (see upper tracing) and with negative atrial pressure. The centre tracing shows signs of arrhythmias possibly associated with a sinus arrhythmia and this is reflected in the amplitude of the ventricular tracings and changes in the conformation of the pulmonary artery pressure waves.

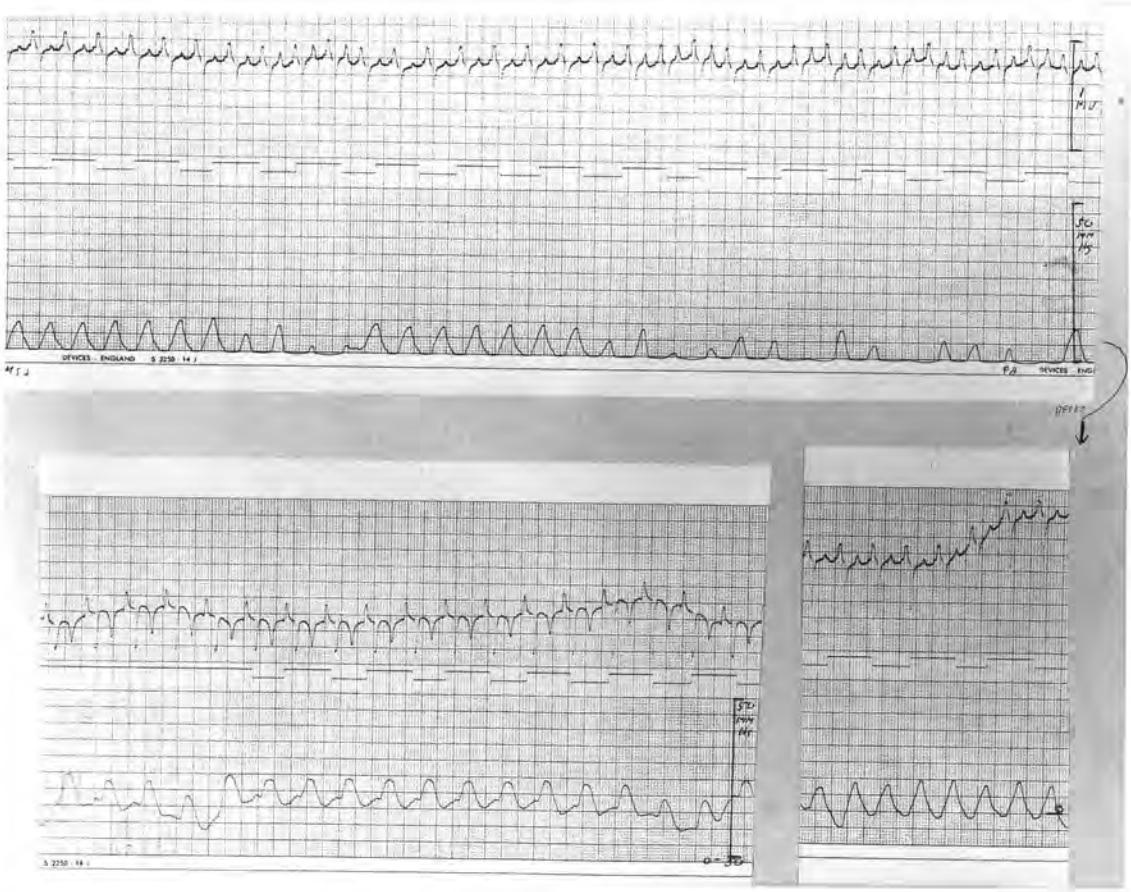


Plate 9: Electrocardiogram of sable bull with adrenaline infusion for urea, blood urea nitrogen and lactate estimation. In this animal an alpha-adrenergic blockade was induced with phenoxybenzamine hydrochloride, resulting in an extremely low pulmonary artery pressure; in sharp contradistinction to the previous sable. As a result, there was an increased cardiac output and a rapid heart rate. The heart shows evidence of sinus tachycardia due to diminished vagal tone augmented by increased sympathetic tone. There are normal sinus P-waves at a rapid rate with a beat exceeding 100 per minute. This configuration is also seen in hyperthermia, the sinus rate tending to increase by some 8 beats per minute for every degree rise in body temperature. It is also seen where there is a diminution of oxygen saturation (Schamroth 1975).

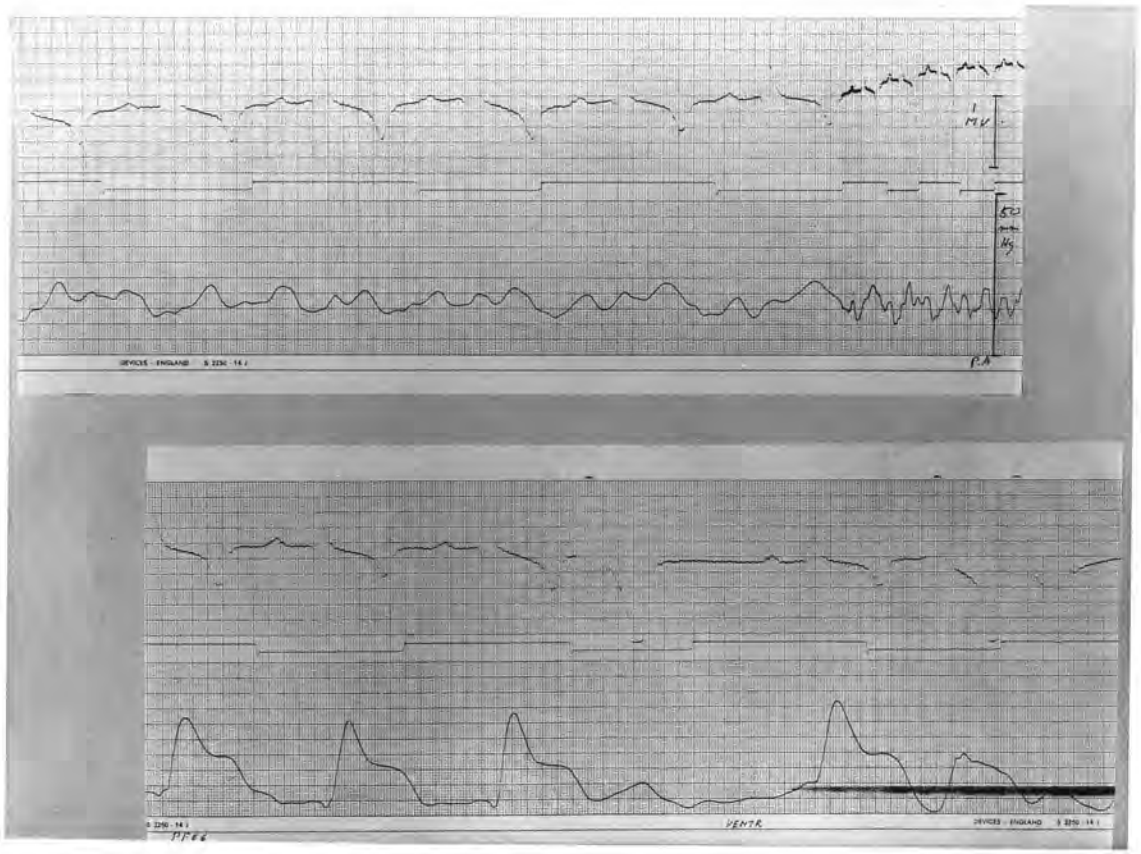


Plate 10: Electrocardiogram of eland bull 30 minutes after forced exercise over 2 km distance. Here we have a sinus bradycardia at a rate of approximately 60 per minute. The sinus bradycardia is characteristically accompanied by a sinus arrhythmia. In the lower tracing, one notices an ectopic beat followed by a refractory period with, however, little change in the blood pressure. The upper tracing shows a characteristic vagal escape showing that the bradycardia is an expression of increased vagotonia. This phenomenon has previously been seen in sympathetic blockade as in the administration of beta receptor blocking agents. The normal configuration of the QRS-complexes suggests a sinus tachycardia rather than a ventricular escape rhythm. On the other hand, the rapid beats at the termination of the lower tracing and the reflected fall in the ventricular pulse pressure suggests a ventricular abnormality and therefore may be an escape rhythm of ectopic origin.

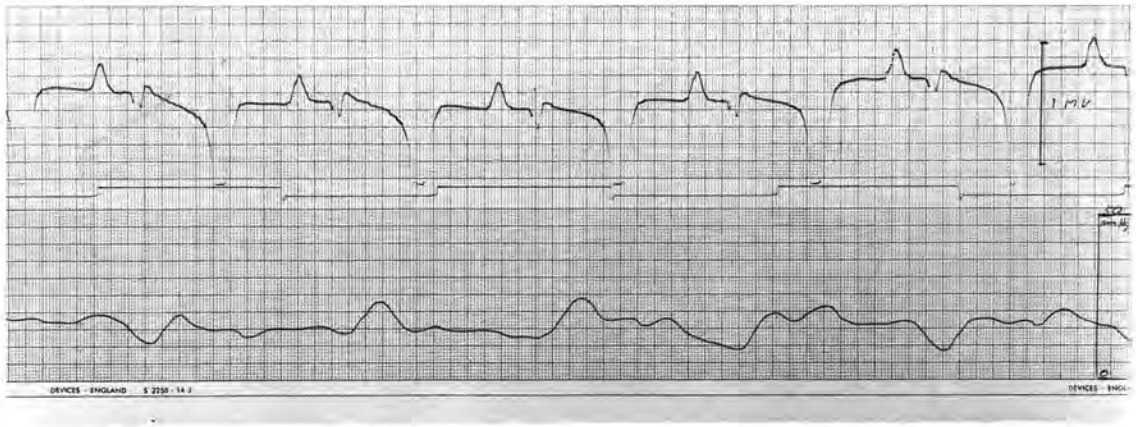


Plate 11: Electrocardiogram of eland bull 30 minutes after forced exercise over 2 km distance. The significance of this electrocardiogram tracing is uncertain. We have here a very strongly developed P-wave with a low voltage QRS-complex. This is followed by an inverted T-complex and a greatly prolonged ST-complex. This manifestation may be due to a combination of a hypocalcaemia and a hyperkalaemia. Vagal hypertonus may be inducing a conduction block. Marked T-wave changes is thought to indicate myocardial disease.

CARDIAC OUTPUT

INTRODUCTION

ONLY a limited number of cardiac outputs have so far been determined. This was due to considerable problems with regard to the catheterisation mechanisms evolved for human use (now modified) and recording of cardiac pressure waves through this instrument.

MATERIALS AND METHODS

A sufficient number have, however, been determined (approximately 12 successful estimations) to confirm the marked lowering of cardiac output in the stressed animals. The method of measuring cardiac output is described in detail in Chapter Two.

RESULTS

MEASUREMENTS indicated that the output in medium-large animals such as zebra and sub-adult eland run at about 10 to 13 litres per minute immediately on capture, or in sable immediately after immobilisation. Soon after capture the output fell to approximately normal resting values of some 9 to 11 litres per minute. Thereafter, as the effects of stress became apparent, the output fell steeply while the pulse rate rose. An output of 5 litres per minute (6,3 and 5,7 in two zebra), normal for man, should be

considered very low for animals weighing some 250 kg or more. In fact, levels considerably lower than this were recorded in acidotic animals. One eland bull with a cardiac defect registered only two litres per minute about one hour after capture. The latter was not suffering from lethal capture stress, as all eland recovered including those run without treatment, i.e. as controls. The cardiac outputs of the original zebra in the Kruger National Park which suffered from lethal capture stress, i.e. fatal when not treated, were not measured.

There was also evidence of a considerable fall in cardiac output in those animals that showed a rise in pulmonary artery pressure. The reasons for this have been discussed under the section dealing with the fall in systemic pressure and were probably mainly associated with a fall in pulmonary blood pressure perfusion. At the same time, the effect of pH on the cardiac musculature must be taken into account as also the effect of the high potassium levels on the heart. In the sable antelope, where the stress was induced by low level adrenaline infusion, the cardiac output could be restored by the infusion of phenoxybenzamine hydrochloride. As the systemic blood pressure was restored by bicarbonate infusion in acidotic animals, it seems likely that the cardiac output is also restored by this procedure, particularly where the heart is affected mainly by the acidaemia.

The cardiac output measured on six eland immediately after exercise and 30 minutes later was analysed statistically

(Table 11). The t-test for paired observations was made

Table 11: Cardiac output in eland immediately after forced exercise and thirty minutes later, in l/min.

eland no.	immediately after exercise	30 minutes later
3	2,9	2,1
4	7,0	4,5
6	12,7	14,9
8	16,9	6,5
9	3,9	2,5
10	5,2	5,0
average	8,10	5,92

to compute the difference or degree of drop between the first time and 30 minutes later. The result was as follows: $t_5 = -1,238$ not significant at 10 percent ($n = 6$).

Discussion

THE small sample size enabled no statistically significant result to be realised from this analysis. Direct observations may be made from the table of raw data above and evaluated on its face value, but clearly further work has to be done in this field. The cardiac output appears to drop on average, over a period of 30 minutes, from 8,10 to 5,92 litres per minute.

It appears, however, that the cardiac output may be used as a measure of the intensity in the acute phase of capture stress. It further appears to be associated with a fall in pH affecting the heart output in two ways:-

(a) A direct effect on the cardiac musculature as well as through depression of calcium ions, associated with a rise in potassium ions due to intra-vascular haemolysis and degenerative changes in skeletal muscle fibres, and (b) by an indirect effect on the alpha receptors of the pulmonary vasculature.

We may, therefore, have a double effect where the increase in pulmonary artery resistance and the associated rise in pulmonary artery pressure causes an increased work-load on the right heart, which is in turn weakened by excess hydrogen ions, excess potassium (Chapter Thirteen) and possibly depression of calcium ions by lactate.

Neither PO_2 nor PCO_2 appear to be causative factors in the actual circulating blood as the levels there are normal, although gross abnormalities are found in capillary blood. A high sympathetic tonus makes the low venous return as a causative factor unlikely; in any case as venous pressures are normal. The cardiac output is greatly increased when alpha receptors are blocked by phenoxybenzamine hydrochloride infusion at low levels (Chapter Eleven).

The fall in systemic blood pressure associated with a lowering in cardiac output must cause considerable decrease in perfusion

of blood in organs such as liver and kidney. The former is associated with the metabolism of pyruvic acid and lactic acid and the lack of function of this organ is indicated by the inability of the severely stressed animal to restore the blood pH to normal levels without assistance in the form of alkaline infusion (Chapter Four). Lack of function of the kidney under these circumstances is illustrated by the rise in metabolites in stressed animals (Chapter Eleven).

CHAPTER SIX

BLOOD PRESSURE

PULMONARY HYPERTENSION

INTRODUCTION

CHANGES in blood hydrogen ion concentration was shown to effect pulmonary vascular resistance and the capacity of the pulmonary vascular bed, a fall in hydrogen ion concentration producing a pulmonary vasodilatation (Enson, Giuntini, Lewis, Morris, Ferrer and Harvey 1964). Previously the role of nervous factors in pulmonary vasoconstriction had been doubted and the changes in pulmonary artery pressure which resulted from immersion cooling ascribed to reduced cardiac output (Kuhn and Turner 1959). Conversely it has been demonstrated that the pulmonary vascular tree is under the control of the autonomic nervous system (Fishman 1964). Furthermore the vascular reflex has been shown to be mediated through the sympathetic nervous system (Hyman 1966), and to be transmitted at least largely by alpha adrenergic receptors (Barwinsky and Reyes 1966, Ingram, Szidon, Skalak and Fishman 1968).

Chronic left heart failure has also been shown clinically to be frequently accompanied by increased pulmonary resistance. The increase in pulmonary resistance apparently preceeds

that of the increase in peripheral resistance (Riordan and Walters 1969).

Experiments on dogs involving total body cooling showed that moderate cooling resulted in a significant rise in pulmonary vascular resistance affecting both the venous and arterial sides of the circulation, but with a greater effect on the venous side (Stern and Braun 1970). Alpha-blockade reduced the vasospasm on the venous side, but on the arterial side only in part. It was concluded that active vasoconstrictor changes were induced by hypothermia.

Alpha-blockade under these circumstances results in improved cardiac function and increased cardiac output. The relief of post-capillary pulmonary vasoconstriction increases the venous return to the heart with improved coronary and cerebral circulation. The lowered resistance of the pre-capillary vessels increases the cardiac output and reduces the work load of the heart.

Alpha-blockade induced in patients suffering from shock of various origins such as sepsis or hepatic disease, and showing clinical evidence of coma and anuria, resulted in improvement of the condition in a proportion of cases (Fromm and Wilson 1969). It was noted that in three patients with pulmonary oedema, alpha-blockade resulted in dramatic disappearance of the rales in a few minutes. This suggests that the observation of Stern and Braun (1970), that the vasomotor changes induced by hypothermia is greater

on the venous side, applies to vasoconstriction induced by methods other than hypothermia, and in fact obtains in clinical cases of pulmonary oedema.

The experiences reported above illustrate the necessity of maintaining adequate blood flow and the importance of tissue perfusion, rather than blood pressure, in maintaining both the integrity of organs and that of the circulation. It has been pointed out (Anderson, James, Bredenberg and Hardaway 1967), that critical reduction in effective blood flow is not necessarily reflected in systemic cardiac output on account of aterio-venous shunts resulting in a hyperkinetic systemic flow without effective perfusion of vital tissues such as the liver.

Materials And Methods

Eight zebra and eleven wildebeest were used. The methods of inducing stress and subsequent measurements are described in Chapter Two. Blood pressure measurements were taken from the anterior vena cava, right atrium, right ventricle, pulmonary artery and posterior aorta.

The location of the tip of the catheter and therefore the location being monitored was ascertained by means of the characteristic wave pattern exhibited by the various anatomical areas mentioned above as recorded on the blood pressure module. The differences can be seen clearly in Plates 12 and 13. The main differences are, in effect,

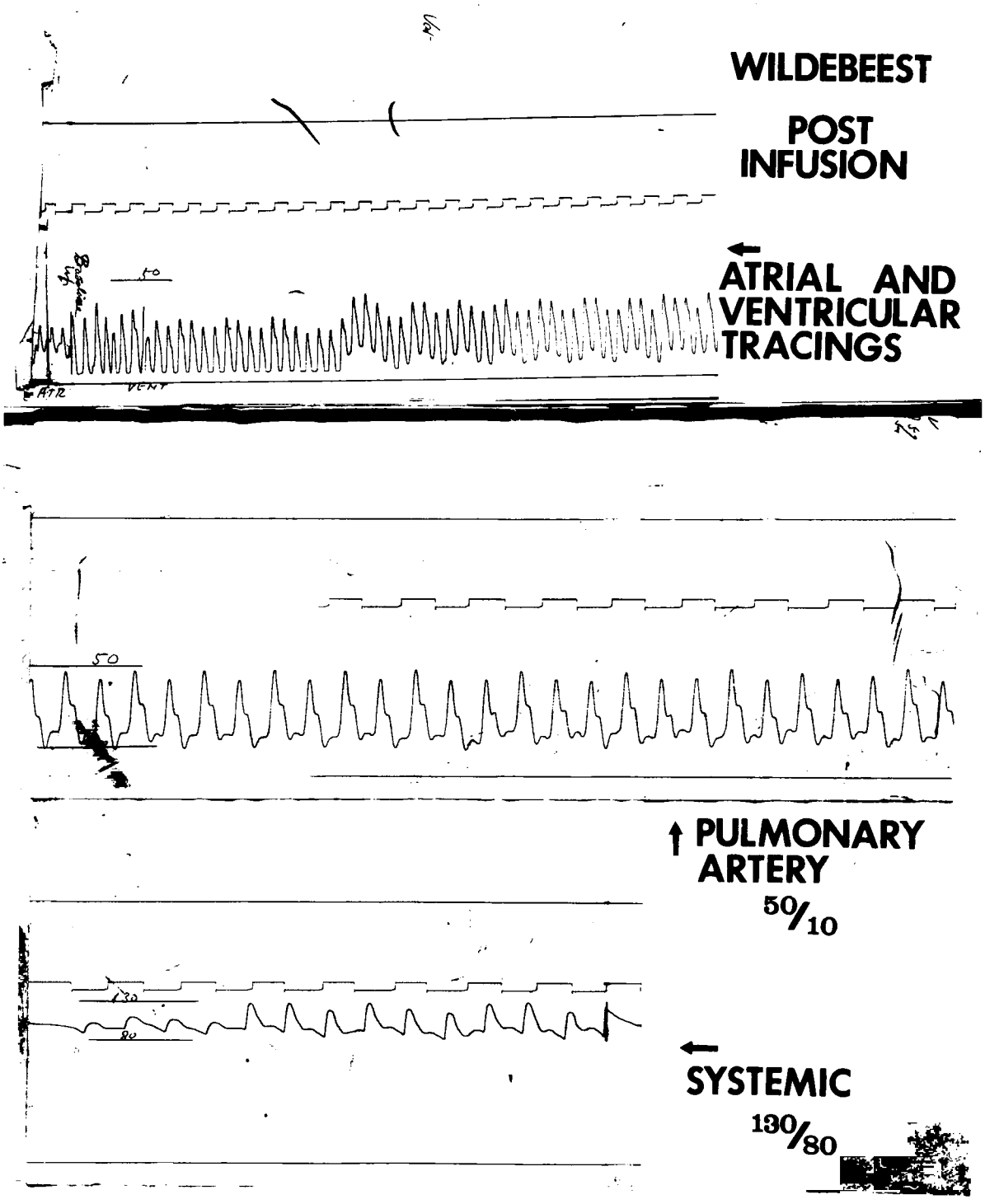


Plate 13: Blood pressure tracings in blue wildebeest after exercise post bicarbonate infusion therapy.

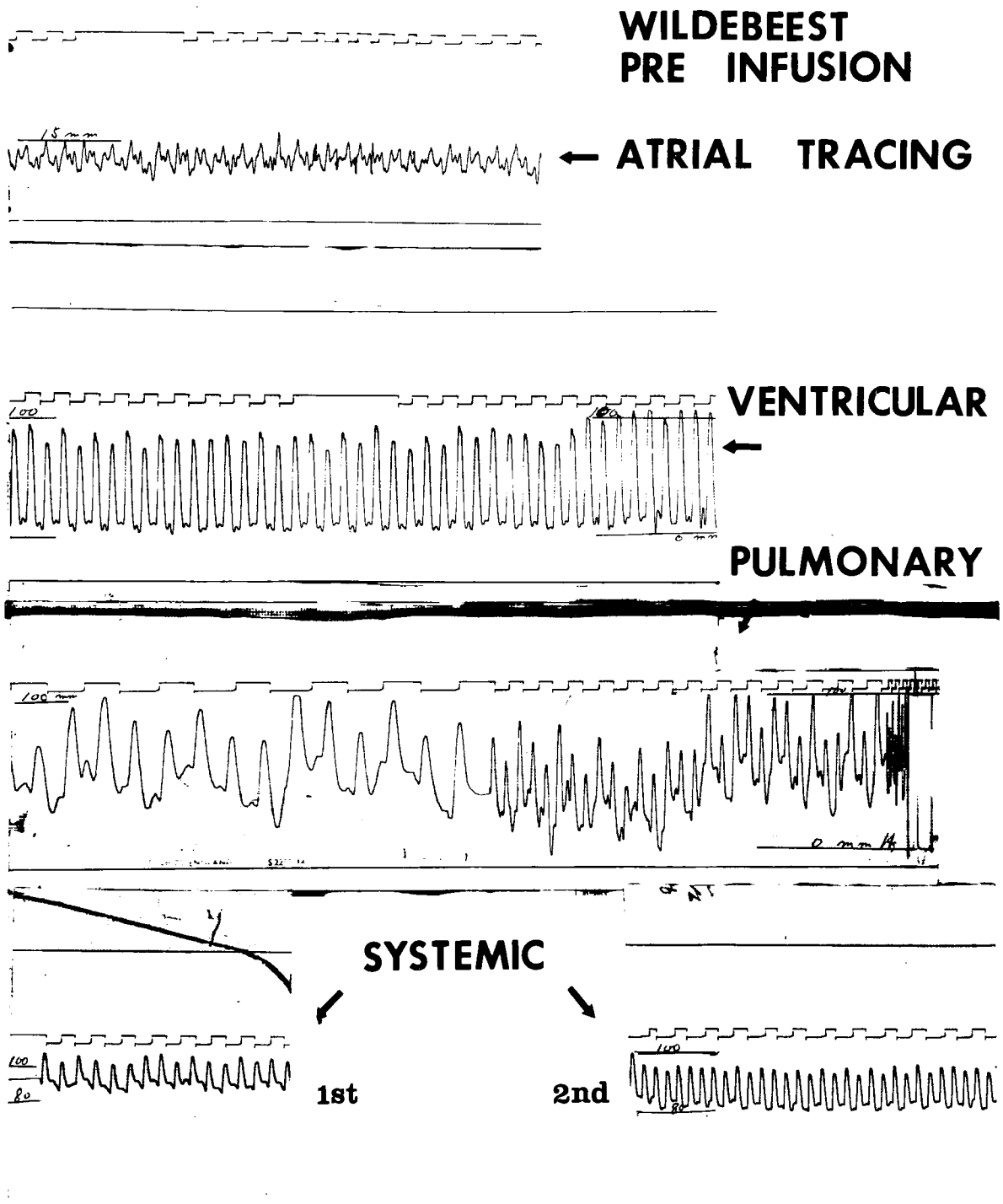
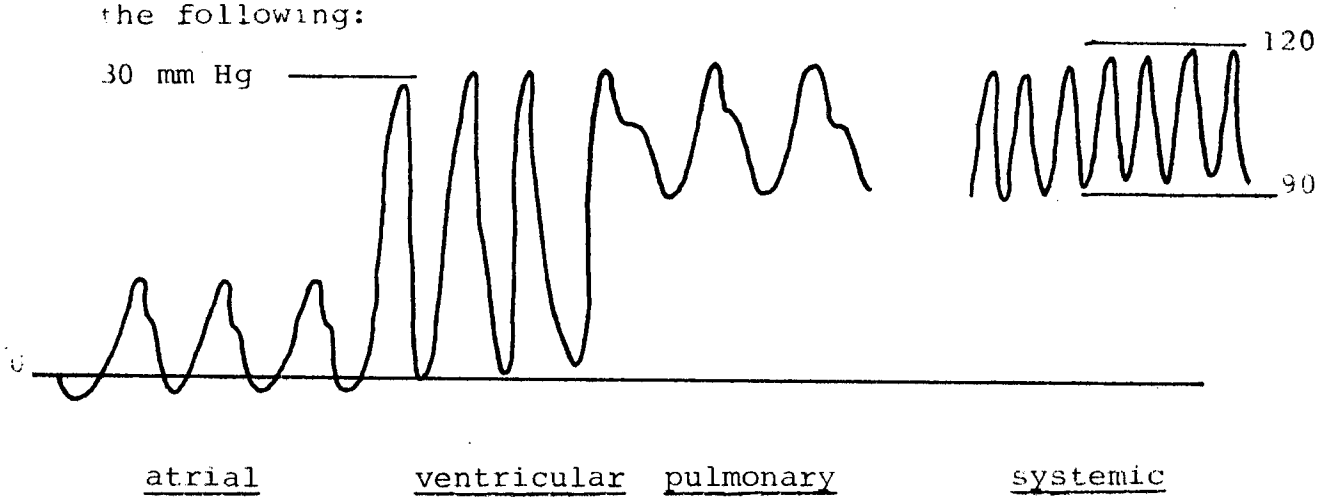


Plate 12: Blood pressure tracings in blue wildebeest after exercise prior to bicarbonate infusion therapy.

the following:



The tracings demonstrate distinct changes in blood pressure that occur as a result of the stresses to which the animals were subjected. Treatment consisted of an infusion made up as described (Chapter Two).

Results

The exact levels of pulmonary arterial pressure are difficult to ascertain owing to muscular movements of the captured animals as also dyspnoea associated with acidaemia. Peaks up to 100 mm Hg were recorded in several instances. The trend of the blood pressure tracings was a decrease in systemic, and an increase in pulmonary artery pressure (Fig. 41).

In some of the animals captured an apparently low systemic pressure and high pulmonary artery pressure was evident immediately on capture (Fig. 42). In these cases further deterioration occurred during the interval between capture and treatment.

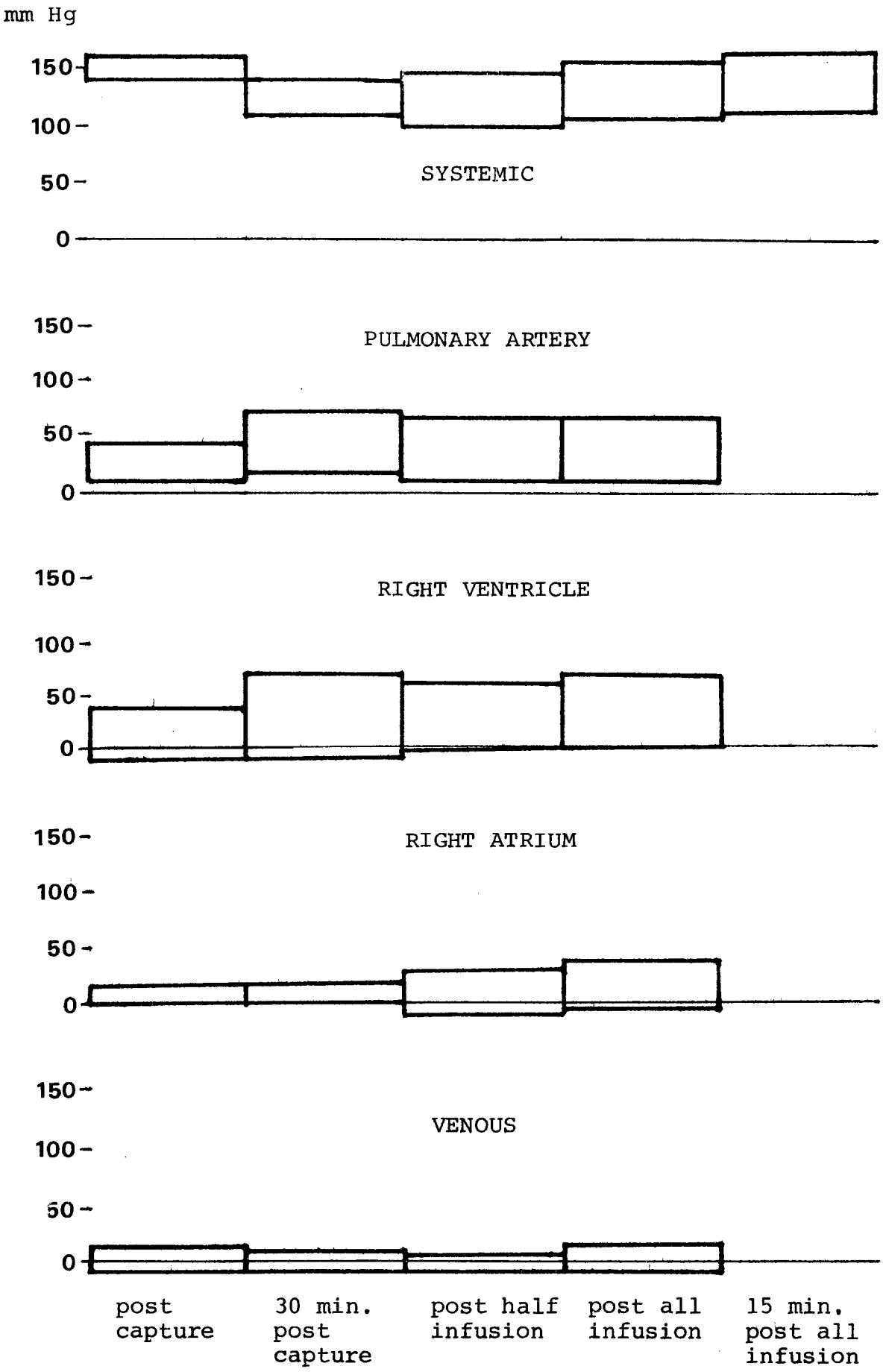


Figure 41: Blood pressure changes in zebra before and after bicarbonate infusion therapy.

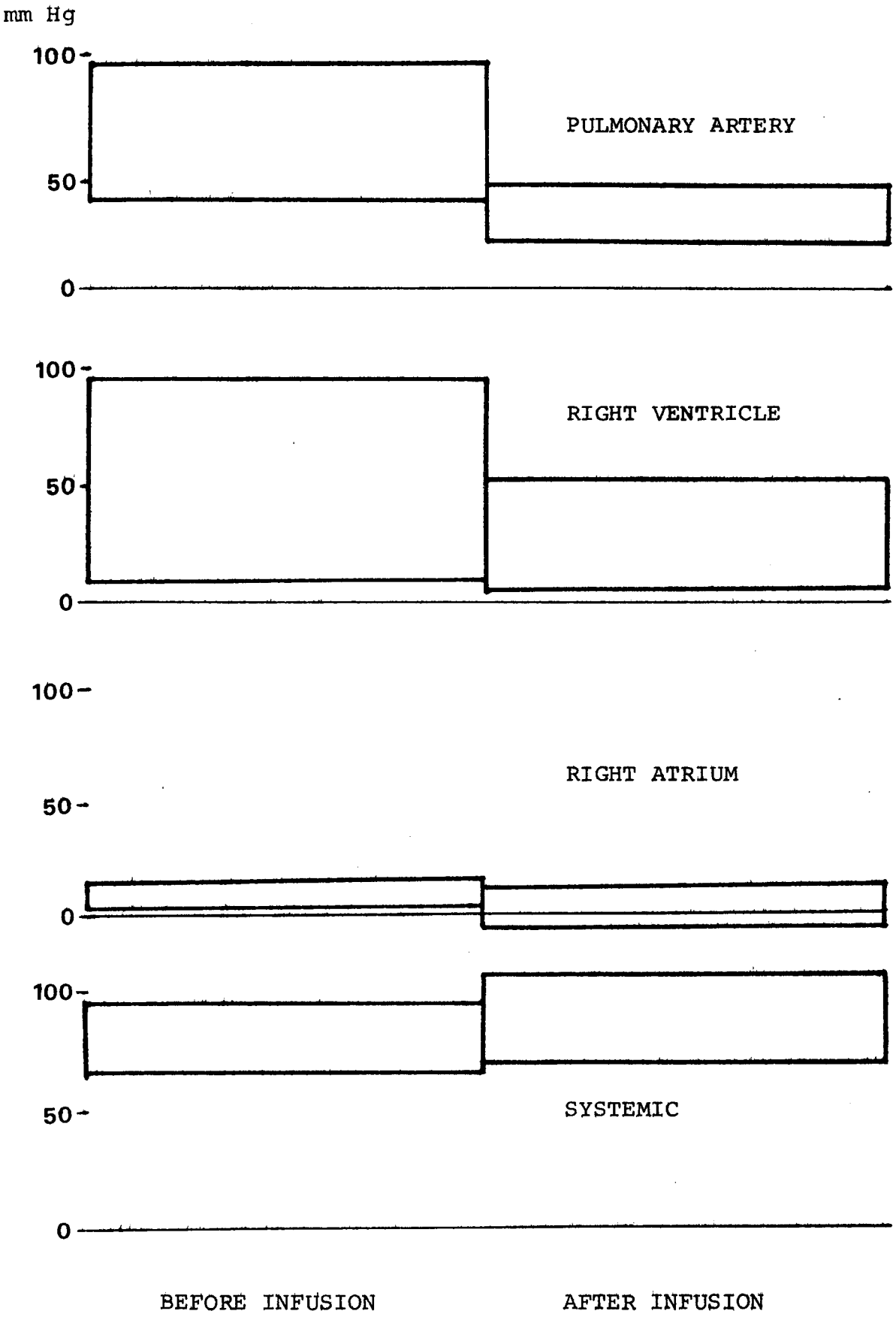


Figure 42: Blood pressure changes in blue wildebeest before and after bicarbonate infusion therapy.

The mean value for all animals for pulmonary artery pressures, taken about 30 minutes after capture was 70/19 mm Hg, falling to 62/12 mm Hg five minutes after infusion of the bicarbonate solution.

The fall in pulmonary artery pressure after the infusion was, in all cases, accompanied by signs of clinical improvement. There was a reduction in dyspnoea and tachycardia, a normalisation of systemic blood pressure, and an increase in alertness.

An apparent fall in systolic pressure was sometimes evident without a comparable fall in diastolic pressure. The cause of this change was a reduced pulse pressure. This reduced pulse pressure was not an artifact due to the location of the arterial catheter, as the arterial catheter is not normally moved from its position during the experiment.

A reduced pulse pressure was a constant phenomenon observed in most if not all the animals subjected to forced exercise. Prior to the direct measurement of the blood pressures, a reduced pulse pressure was recorded by palpation and was related to a decrease in the amplitude of the heart sounds as determined by auscultation.

Pulmonary artery pressure was not in all cases high immediately after capture, but tended to rise during the first 30 to 60 minutes after capture (Table 12). The values soon after capture are sporadic owing to the difference in

Table 12: Blood pressure curves in zebra and wildebeest after forced exercise (averages of 19 animals)

	on capture	after 30 min.	5 min. after infusion
venous			
zebra	8/-6	8/-6	12/6
wildebeest	-	-	-
atrial			
zebra	8/0	8/0	20/4
wildebeest	13/2	-	12/-6
ventricular			
zebra	30/-6	70/-6	70/-5
wildebeest	98/15	-	48/3
pulmonary			
zebra	40/15	70/19	62/12
wildebeest	98/40	-	45/28
systemic			
zebra	154/130	130/110	155/112
wildebeest	98/65	-	105/70

times taken for the catheterisation procedures.

The rise in pulmonary artery pressure is accompanied by a further fall in blood pH in zebra (Table 13) and a rapid deterioration of the clinical condition during the first 60 minute period. In some cases, recording was complicated by bloat in the captured wildebeest.

Table 13: pH values in zebra and wildebeest after forced exercise (average of 19 animals).

immed. after capture	30 min. later	after half infusion	after all infusion
zebra			
6,77	6,69	7,13	7,29
wildebeest			
6,95	7,18	7,58	7,62
S_x			
zebra			
0,18	0,13	0,11	0,12
wildebeest			
0,11	0,02	0,03	0,03

The changes that occur in right atrial pressure were more variable. Any rise in right atrial pressure, however, causes a reduction in venous return. An increase in atrial pressure causes a back-loading effect on the systemic circulation distending the veins and other vessels rather than permitting this blood to flow to the heart. It is stated that if the right atrial pressure was to approach the mean systolic pressure the return of blood to the heart to the peripheral circulation approaches zero and the cardiac output becomes zero (Guyton 1966). This is, however, an understatement as the right atrial diastolic pressure needs, in fact, to increase only a few mm Hg to decrease the venous return and to cause back pressure on intestines, liver, abdominal and other organs. Such an increase in the venous pressure and in the diastolic atrial pressure was not recor-

ded, although in the wildebeest the diastolic venous pressure on capture was recorded as several mm lower than in the pre-infusion animals.

DISCUSSION

IT may be postulated that the lung is one of the target organs for stress in zebra, and possibly also in antelope such as wildebeest. It has been determined that the basic haemodynamic disturbances caused by stress and hypotension differ among the various species. In the dog the intestine appears to be the most sensitive organ, becoming engorged with blood as a result of hypotension and shock; in man the most marked changes appear in the kidneys, and in the rabbit it also appears that the lung is the primary target organ (Lillehei, Longerbeam and Bloch 1963).

Changes in the lung occur also in dogs which, when deprived of normal oxygen tension during anaesthesia, exhibit an increased pulmonary vascular resistance which is associated with a decrease in cardiac output. The latter fell from 2,45 litres per minute to 1,95 litres per minute, while the pulmonary artery pressure rose from 14 to 22,5 mm Hg (Barwinsky and Reyes 1966). When the dogs were treated with an alpha adrenergic blocker, no rise in pulmonary vascular resistance or in pulmonary artery pressure occurred, suggesting that the increase in vascular resistance was mediated through alpha-adrenergic receptors. It was pointed out that the phenomenon was due to adrenergic discharge

rather than any direct effect of oxygen lack (Barwinsky and Reyes 1966).

Similarly, after capture, our animals showed normal blood PO_2 and PCO_2 levels (Chapter Four) and there is a considerable drop in cardiac output under the conditions of stress during which the pulmonary artery pressures were monitored.

Arteriospasm occurs in man as a result of massive adrenergic discharge. Normal values for circulating catecholamines are less than one microgram per litre of blood volume. These levels, which are not increased during the minor surgical procedures rise during radical surgery, such as extra corporeal bypass, to values of 10 to 30 µg/l (Hammelberg, Sprouse, Mahoffay and Richardson 1960, Rosenburg, Lillehei, Longerbeam and Zimmerman 1961, Indeglia, Levy, Lillehei, Todd and Lillehei 1966). Adrenaline infusion at a rate sufficient to cause tachycardia and cardiac arrhythmias had only very minor influence on the pulmonary blood pressure in immobilised sable antelope.

Further evidence may indicate that pulmonary vascular resistance is associated with sympathetic discharge. Increased pulmonary vascular resistance in dogs induced by hypothermia and cooling of the pulmonary circulation, could be largely abolished by alpha-blockade (Stern and Braun 1970). Also pulmonary oedema (a constant major cause of death in the untreated zebra) was abolished by means of alpha blockade in man (Fromm and Wilson 1969). More recently, an incr-

ease in pulmonary vascular resistance has been established as a result of stimulation of the upper thoracic chain in *Papio* species using isolated lung lobes (de Burgh Daly, Ramsay and Waaler 1975). It was noted that the greater degree of anxiety and excitement of the animals during capture and anaesthesia, the less the responsiveness of the pulmonary vascular bed to nerve stimulation, due apparently to the transmitter concerned which had been partially exhausted by the initial excitement of the animals.

From the results of treatment with bicarbonate infusion, and the evidence cited above, it appears probable that the rise in pulmonary artery pressure, reported here, is partly due to the action of low pH values. The fact, however, that the pulmonary artery pressure failed to return to the generally accepted normal levels after infusion suggests that the implication of another factor or factors, possibly is sympathetic in origin.

SYSTEMIC BLOOD PRESSURE

Results And Discussion

THE appreciable fall in the systemic blood pressure which was reported as occurring in the animals captured in the Kruger National Park, was not seen to the same extent in the animals on the track at Percy Fyfe Nature Reserve. The fall in the systemic blood pressure occurs *pari passu*

with the rise in the pulmonary artery blood pressure.

The cause of these phenomena may be the following:-

(a) A damming back of the circulation due to pulmonary vasospasm resulting in reduced perfusion and a lowered return of blood to the left heart;

(b) a right heart dilatation causing a reduced cardiac output into the pulmonary circulation. Note that a dilatation causes not only an increase in the residual blood in the ventricle and lowered stroke volume, but an incompetence in the valves resulting in back-flow of blood;

(c) a peripheral vasodilatation and pooling of blood in the extremities and in the splanchnic area;

(d) fall in the cardiac output *per se* , and

(e) reduced venous return.

Of these, (c) and (e) may be discounted. Venous pressures are normal and there is peripheral vasoconstriction rather than vasodilatation. A reduction in circulating blood volume indicates sequestration of parts of the circulation apparently due to constriction as a result of sympathetic hypertonus. This state of affairs is unaffected by infusion of adrenaline but radically changes after the administration of phenoxybenzamine hydrochloride (Chapter Eleven).

The mechanism mentioned under (a) and (b) may have relevance particularly in zebra. In these animals, considerable cardiac dilatation was observed on auscultation. Dilatation especially of the right side of the heart was evident in

animals that died after capture. It is probable that pulmonary vasospasm is directly responsible for a lowering of the lung perfusion and therefore a reduction of blood flow to the left heart (Chapter Five).

A fall in cardiac output during the onset of capture stress has been established. This is associated with tachycardia and signs of central depression. This fall in cardiac output may be due indirectly to lack of pulmonary vascular perfusion as outlined above, or indirectly to lack of cardiac contractility or the inotropic cardiac effect The cause of this is likely to be excess potassium (Chapter Thirteen) and the action of the low blood pH on the cardiac musculature. The latter appears to be both a direct action on the cardiac muscle and has indirect actions via the cardiac centres, and to the depression of calcium ions by circulating lactate.

Six tsessebe bulls were measured at varying times after exercise on the exercise track described in Chapter Two. All systemic blood pressures were high, ranging from 150/100 to 170/100 with very little variation from animal to animal (Table 14). There was no statistically significant correlation between blood pressure and duration of handling after capture, in this experiment.

Three separate correlation coefficients were calculated. The results were as follows:

Minutes after exercise with systolic pressure: r = 0,02097

Table 14: Systemic blood pressure measurements on tsessebe after forced exercise.

animal no.	min. after exercise	mm Hg
1	40	160/95
2	20	150/100
3	24	170/100
4	5	165/105
5	11	155/90
6	5	160/97
average		160/98

Minutes after exercise with diastolic pressure: r = -0,2102

Systolic pressure with diastolic pressure: r = 0,4147

None of these results were statistically significant. The partial correlation revealed the following:

$t_{23 \cdot 1}$ = 0,4287 which was not significant.

From the above and from Table 14 little variation can be seen in systemic blood pressure. As can be expected there is some correlation between diastolic and systolic blood pressure after exercise, but the fluctuations are too large for any significance.

The infusion of bicarbonate in the zebra and wildebeest is effective in restoring the systemic blood pressure virtually to near normal levels. It may be noted that the pulmonary artery blood pressure is not completely restored to

normal levels by this treatment and it has been postulated (see previous section in this chapter) that a part of the raised pulmonary artery pressure is due to the direct effect of the high level of hydrogen ions and partly due to sympathetic discharge acting in alpha receptors. It is likely, however, that any residual restriction of blood flow due to the latter, is insufficent to affect the cardiac output.

CHAPTER SEVEN

BODY TEMPERATURE AND VENTILATION

BODY TEMPERATURE

Materials And Methods

Body temperature was taken by a standard clinical thermometer inserted completely into the rectum of zebra and antelope over one minute at regular intervals of 30 minutes. Where cardiac output measurements were made, the temperature was also taken inside the pulmonary artery so measuring the temperature of the mixed venous blood from the entire body and therefore the central or core temperature which has been shown to bear a constant relationship to the rectal temperature (Bligh and Harthoorn 1965).

Rectal temperature was taken routinely in all test animals. Records of particular interest were those taken in six tsessebe and eight eland immediately after exercise and every 30 minutes, and in 18 blesbok after runs of different lengths.

The object of the temperature measurements was to determine whether there was validity in the theory of *malignant hyperthermia* as a factor in capture myopathy, as suggested (in discussion) at the 1973 South African Veterinary Association

congress.

RESULTS

IN blue wildebeest the body temperature showed a fall which commenced immediately after capture and continued throughout the time of the experiment (Fig. 43).

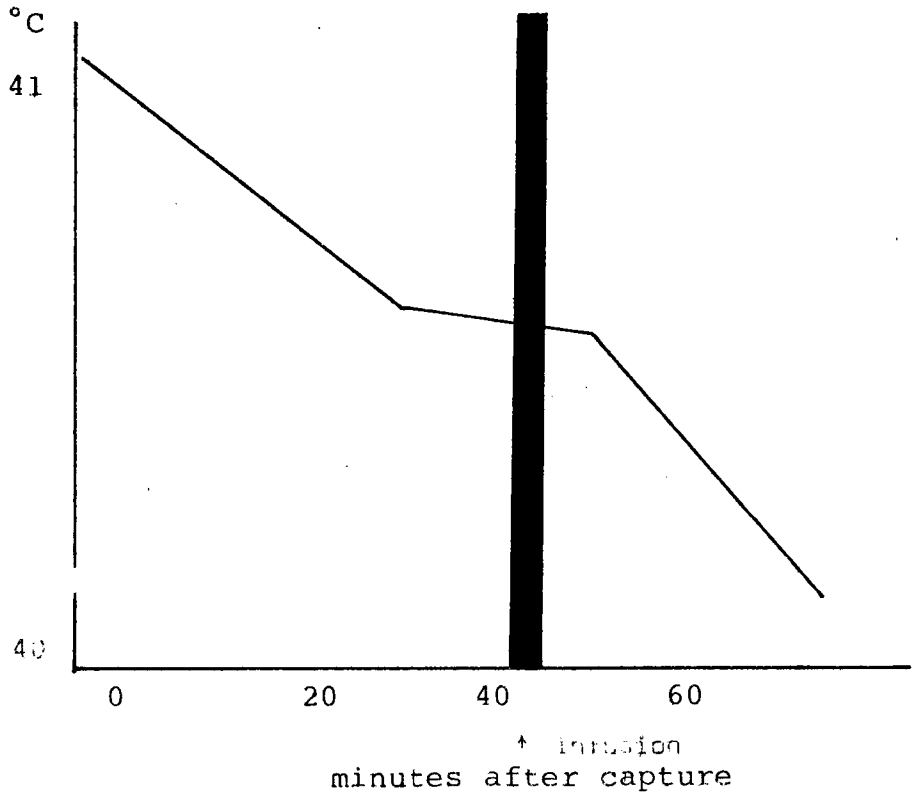


Figure 43: Body temperature of wildebeest after capture.

Zebra showed a rise in body temperature during the first 30 or 40 minutes after capture (Fig. 44 shows the average of eight animals). The temperature in zebra may have been influenced by several possibilities:

(a) The necessity to tie the mouth of the zebra and therefore causing a certain amount of interference with breathing;

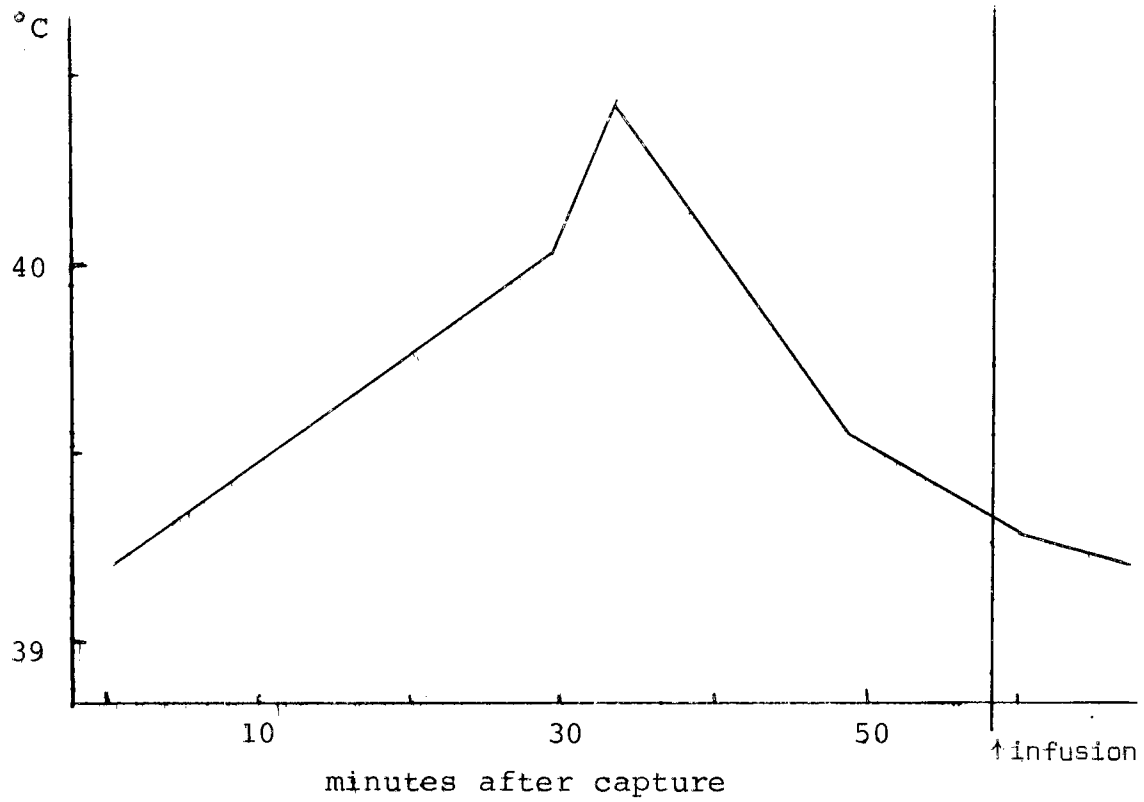


Figure 44: Body temperature in zebra after capture.

(b) the reliance of the zebra on sweating rather than on panting;

(c) interference with the sweating mechanism while the animal lay in lateral recumbency on a plastic sheet;

(d) the possibility that sweating is in some circumstances at least, a less efficient cooling mechanism than that of panting, and

(e) that the thermoregulation of the wildebeest is better developed than that of the zebra.

The temperature rise recorded in zebra is small, barely exceeding 40 °C and less than 1° above capture levels.

More pertinently, in the context of possible malignant hyperthermia, the temperature in all animals fell to approximately normal levels at 60 to 90 min. after capture.

Conversely, one of the sable infused with adrenaline showed a persistent rise in body temperature is spite of wetting the body surface and ventilation by an electric fan held about one metre from the animal.

Eland body temperatures rose significantly within 30 minutes after exercise as did the zebra, but rarely exceeded 41 °C. A straighforward correlation coefficient was calculated for the set of values immediately after exercise and the set of peak values within 30 minutes. The coefficient was as follows: $r = 0,7444^{**}$ $(n = 8)$.

The t-test for the paired observations as detailed in Table 15 was made and the result was $t_7 = 4,610^{***}$. There was a high degree of significant difference between values after exercise and peak values within 30 minutes. The temperature rose significantly after exercise in all cases, and there was a highly significant positive correlation between both sets of values.

A correlation was calculated between the body temperature values in tsessebe and the distance they ran. The result was as follows:

$r = -0,6386^{*}$ (one-sided) $(n = 6)$

There was a reasonable degree of significance in the corr-

Table 15: Body temperature in eland after forced exercise.

eland no.→	1	3	4	6	7	8	9	10
immed. after chase (°C)	40,7	39,6	39,9	39,5	37,2	38,9	38,6	38,2
peak value (°C)	41,6	40,1	40,3	40,0	39,4	40,5	40,1	40,4

average: immed. after - 39,08, peak value - 40,3

elation of these two factors. The negative correlation showed that the body temperature was lower in those animals that had run the furthest (Table 16).

Table 16: Body temperature in tsessebe compared to distance chased.

animal. no.	distance run (km)	body temperature (°C)
1	1,875	40,6
2	2,0	40,7
3	1,875	43,0
4	1,750	42,7
5	2,0	40,3
6	2,0	41,7

There was scant evidence of the occurrence of a sustained temperature rise or of malignant hyperthermia in animals that were not immobilised.

DISCUSSION

IT has been pointed out (Nahas, Ligou and Mehlman 1960) that hypercarbia is a potent stimulus for catecholamine release from the adrenal medulla and the extramedullary chromaffin tissue. The rise in body temperature is ascribed to metabolic changes and the rapid utilisation of muscle glycogen (Hall, Lucke and Lister 1975). On the other hand, the porcine malignant hyperthermia syndrome could be controlled with alpha-blockade (Lister, Hall and Lucke 1975) although the sable showed similar rises in body temperature when adrenaline was infused with and without the alpha-adrenergic blocker phenoxybenzamine hydrochloride.

Conversely, Lister *et al* (1975) point out that once the initial muscle stimulation reaction is established, it is almost impossible to create the necessary degree of alpha-block in the presence of the concentrations of adrenaline, the agonist responsible for the mobilisation of energy substrate.

Intensive muscle tension was observed in the sable during incomplete immobilisation and this was independent of the infusion. And the rise in body temperature commenced without the addition of exogenous adrenaline. We may therefore have the possibility of a malignant hyperthermia-type syndrome in some of the more nervous species of animals such as sable, under conditions of chemical or even manual restraint.

The role of elevated body temperature as a possible lethal factor in animals chased over long distances (e.g. exceeding 30 miles in Rhodesia - Garstang†, pers. comm.) has yet to be investigated. These animals survived, however, while the same species (tsessebe) chased only a short distance to permit remote injection from a helicopter, suffered high or total mortality from capture myopathy (Young††, pers. comm.).

The finding that the tsessebe showed a lower body temperature when the distance run was longer compares with the results of the blood hydrogen ion estimation which was also found to be lower in animals that had run a longer distance. This may be due to one or more of three factors:-

(a) The animals become tired and are seen not to exert themselves to the same extent as in the early stages of the chase.

(b) The number of muscles used are reduced while the animals are observed to run approximately in a straight line instead of leaping, turning and twisting as commonly occurs during the early stages, and

(c) glycogen stores in the body will have been reduced.

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†† E. Young, formerly State Veterinarian, Skukuza, presently Transvaal Division of Nature Conservation.

The latter may be of particular importance in relation to both a fall in pH and a rise in body temperature. It has been pointed out (Hall *et al* 1975) that both the rise in temperature and the fall in pH are due to the rapid utilisation of glycogen stores as a result of massive adrenaline secretion. Also that the damage to the muscle (meat) in slaughtered pigs is due to the interaction of a high body temperature and acidity (Lister 1975).

The results suggest that animals should theoretically be captured only after a prolonged, less intense chase which is sufficient to tire and use up glycogen stores without causing strain, intravascular haemolysis, sympathetic hypertonus or excessive hypoxaemia.

This is not easy to accomplish but it has been done during the capture of zebra herds which were driven for distances of some 30 kilometres by helicopter at a fast trot or a slow canter with regular stops for twenty minutes or so during which the helicopter was grounded (Timbavati Nature Reserve - own records).

VENTILATION

PO_2 AND PCO_2

THE respirations of animals at the time of capture are usually deep and relatively slow. More rapid and shallow

respiratory movements tended to develop only later during the period after exercise. The deep respirations coincide with a low oxygen content of the central blood and a higher level of CO_2. During this time the body temperature tended to rise.

As the blood PO_2 level increased and the PCO_2 level decreased, there was a shift toward the more rapid and shallow *panting* which marked the beginning of a fall in body temperature. This respiratory pattern may therefore be referred to as thermal panting, and resulted in depressed PO_2 and raised central PCO_2 values.

These two periods of respiration were associated with different patterns in the systemic blood pressure.

pH

A third period of respiration developed about half to one hour after capture characterised by forced respiration or dyspnoea. This was characteristic of those animals who were unable to raise the pH of the central blood after capture and when the blood pH levels fell in spite of respiratory efforts.

Respiratory Functions

RESPIRATION in animals after forced exercise carries out three important functions. These are:

(a) To restore the blood gas pressure to normal levels;

(b) to restore the acid-base balance, and

(c) to restore normal body temperature.

Functions (a) and (b) are very similar and rely on an adequate gaseous exchange between body and atmosphere. The most efficient method of breathing is therefore adopted, i.e. deep inspirations that make maximum use of the lung capacity, and reduce the anatomical dead-space to a comparative minimum. Function (c) by comparison, relied on a maximum use of dead-space without overventilation and thereby without inducing a hypocapnoea.

Greater fluctuation in blood pressure (and probably also the cardiac output) occurs during the phases (a) and (b) than phase (c). This is perhaps not unexpected in relation to the greater fluctuation of positive and negative pressure in the thorax during the former phases.

Since we are greatly concerned with changes in resistance of the pulmonary vascular bed, both as a symptom of incipient capture shock and as a possible factor in its precipitation, the observed phenomena may bear closer analysis.

Cardiac Effects

Left ventricular output or aortic ejection has been variously described to remain the same or to decrease during inspiration.

This apparent discrepancy may be due to the delicate balance that must exist between left ventricular filling by blood from the pulmonary vascular bed, and left ventricular outflow impedence. Both these factors are liable to individual variation and in increased pulmonary vascular resistance and in dyspnoea, and varying degrees of airway obstruction which are virtually inseparable from normal restraint of hoofed animals, but particularly of zebra - which are devoid of horns and liable to inflict severe bites, both necessitating a firm hold of the snout.

Blood Pressure

THERE was a fall in systemic blood pressure during the time of increase in resistance of the pulmonary vascular bed (as estimated from the increase in pulmonary arterial pressure). This is assumed to be due *a priori* to a a *lowered left ventricular* filling, rather than to a left heart failure as such, although a reduction of left heart output from reduced contractile power may be postulated in the pH range at which we were working; left heart back pressure into the lung may cause lung oedema, irrespective of a rise in pulmonary vascular resistance.

It must be assumed that over any given period and during a steady state, the amount of blood entering the lungs and the output of the left heart are equal.

There are of course the two subsidiary circulations, i.e.

the bronchial circulation and the coronary flow. These two circulations are, however, small and tend to stabilise each other during the phases of the cardiac cycle, the coronary flow occurring at diastole and that of the bronchial flow during systole.

Other Factors

We therefore have a number of interesting and possibly conflicting factors affecting the respiration and the blood pressure. The stimuli to respiration are derived from metabolic factors and from body temperature. Additional factors are blood pressure and sympathetic discharge. Blood pressures are influenced by resistance of the pulmonary vascular bed, by the degree of left ventricular filling, and by the viability of the ventricular musculature, and therefore the cardiac output is a result of metabolic factors including low blood pH, possible reduction in plasma calcium ions, and increase in circulating potassium. Other factors such as histamine release, angiotensins, and dilatation of the visceral vascular bed cannot be excluded.

Exercise

A significant correlation was calculated between respiratory rates and the speed of chase of tsessebe. The coefficient is $r = 0,9739$**(one-sided) or *(two-sided) ($n = 3$), so that as the animal is chased at greater speed, the faster will be its respiratory rate after exercise.

The lack of clear effects associated with reduced circulating oxygen are surprising in that the animals must be running at a pace considerably exceeding the VO_2 maximum (Pugh 1974). This may, however, be ascribed largely to the interval that elapses between the capture and the taking of central blood samples. It should also be noted that in many cases the capillary blood showed a lower O_2 tension than that of the arterial blood, suggesting that at least parts and a probably large proportion of the circulation was excluded from the central blood flow, presumably to augment cerebral blood PO_2 levels.

It has been noted (Pugh 1974) that the effort expended by a leading cyclist as a result of wind resistance is considerably greater than that of the rest of the group travelling behind. It is not impossible that this also applies to groups of animals, not only in relation to wind resistance, but also resistance of high vegetation or grass. Also the postures of cyclists are relatively static in comparison to our animals which have to jump continually over vegetation. The resistance of the vegetation itself, has to be taken into account together with the type of ground (e.g. soft sand). Note that the cycling experiment was performed on a disused air runway and the bicycle tyre pressures were up to 100 lbs per square inch (5,6 to 7,0 kg/cm^2). We note also that squat jumps in marine recruits are more likely than other exercises to result in the liberation of haemin or porphin pigments (Chapter Nine). This may have relevance to the liberation of pigments after a

chase involving unusual leaping over vegetation and heavy exercise of muscles normally only slightly used.

The utilisation and therefore the needed intake of oxygen rises steeply in relation to the speed and type of exercise. The need for oxygen does not rise exponentially with the need for exercise, but geometrically in relation to increased muscle tension. Furthermore, under maximum exercise there is a greatly increased liberation of muscle glycogen together with the involvement of so-called fast-twitch muscle fibres requiring greater quantities of oxygen for its combustion with a tendency to the formation of excessive quantities of lactic acid due to anaerobic glycolysis (Gollnick, Piehl and Saltin 1974). The massive utilisation of muscle glycogen is more apt to occur in untrained animals. It may be interesting to note that a speed of 40 km/h at which the oxygen consumption rose to over 100 percent (in the cycle experiment cited above) is also the speed obtained by antelope and zebra subjected to capture in the wild.

CHAPTER EIGHT

HAEMATOCRIT

INTRODUCTION

THE haematocrit or packed-cell volume is a simple and valuable technique to give approximate indication of several factors such as haemoconcentration. It does not indicate the mass of red cells, but is the volume of erythrocytes expressed as a percentage of the whole blood of a given sample.

MATERIALS AND METHODS

CARE has been taken that the conditions obtaining during the centrifugation of a number of samples was constant. Normally, 2 500 revolutions per minute for 30 minutes or 10 000 for 10 minutes was taken as providing the force required for inducing rapid settling of the erythrocytes. Heparin has been used as anti-coagulant to avoid shrinking of the cells and thus give a low reading. Rapid sedimentation of the erythrocytes can cause further errors and the syringe of blood was therefore thoroughly mixed. During early work and during the slower centrifugation, the haematocrit tubes were capped to prevent evaporation during the 30 minutes required for complete centrifugation. Later,

rapid centrifugation of multiple capillary tubes was used as the method. To permit valid comparisons to be made, blood for haematocrit studies was drawn from animals in the same physiological state, i.e. immobilised or manually restrained, tame or wild, as the haematocrit is readily raised during stages of excitement and reduced by drugs causing splenic enlargement.

The clinical state of the animals was assessed in the interpretation of the haematocrit. Shock, even that occurring after haemorrhage, may give an increased haematocrit and in other forms of shock the haematocrit tends to be substantially increased. The haematocrit has been shown to be high in conditions of adrenergic discharge. Also in stasis of blood from the use of tourniquets, in anhydraemia and dehydration. It is accepted as being low in certain hydraemic states such as those found in pregnancy, although the total mass of red blood cells is in fact normal.

A rise in haematocrit in progressive samples taken by standard means in an animal is a valuable indication of loss of fluid from the circulation. This occurs in conditions of sequestration of blood with resultant capillary damage, in tissue damage, in reduced blood and perfusion pressure, and any condition in which plasma is lost from the circulation.

A total of 2 342 haematocrits has been taken from zebra, wildebeest, sable, eland, tsessebe and black wildebeest.

In general the findings are that:

(a) *Regular* sampling for haematocrits from tamed animals under standard conditions is a useful indication of the general nutritive condition;

(b) sporadic sampling from nervous or wild animals gives widely fluctuating results;

(c) no comparison can be made among animals sampled under differing conditions such as manual restraint and immobilisation, and

(d) sampling at regular intervals from stressed or immobilised animals gives a valuable clinical picture of the circulatory state.

A rise in the haematocrit is associated with a reduced circulatory blood volume and, together with blood volume measurements, indicates conditions that either determine or soon lead to irreversible circulating derangement. A raised haematocrit in itself indicates greater blood viscosity and therefore increased circulation time. If the blood, taken from the peripheral circulation, shows the haematocrit to be excessively raised it indicates peripheral circulatory failure and loss of plasma into the intracellular spaces. Among the stressed animals tested, many showed a rise in haematocrit values indicating at least the commencement of circulatory failure, hypotension, capillary damage, increased vascular permeability and shock.

RESULTS

ZEBRA

HAEMATOCRITS taken throughout the period after capture and infusion indicate certain interesting trends. These are as follows:-

(a) The haematocrit fell soon after capture presumably during dissipation of the intense adrenergic discharge resulting from the action of capture. The haematocrit continued to fall during the ensuing interval up to the time of infusion. This was the time when the clinical condition of the animal and the various physiological parameters indicated a general deterioration of the animal's condition, as evinced by blood pressure, heart rate, etc.

(b) Immediately after infusion, the haematocrit level fell to about 10 percent of the previous value. This is to be expected from the infusion of one litre into animals weighing 200 to 250 kg with a blood volume of some 20 litres, approximately half of which is composed of blood cells.

(c) Shortly after infusion the haematocrit rose. The fact that this was accompanied by a fall in blood PO_2 levels and a rise in blood PCO_2 levels suggests that this rise was due to a return of red blood cells to the circulation (Fig. 45).

(d) More light may be thrown on this question by the measurement of blood volume, but which has not yet been carried out on a routine basis. There are indications, however, that the fluctuations of the haematocrit are corr-

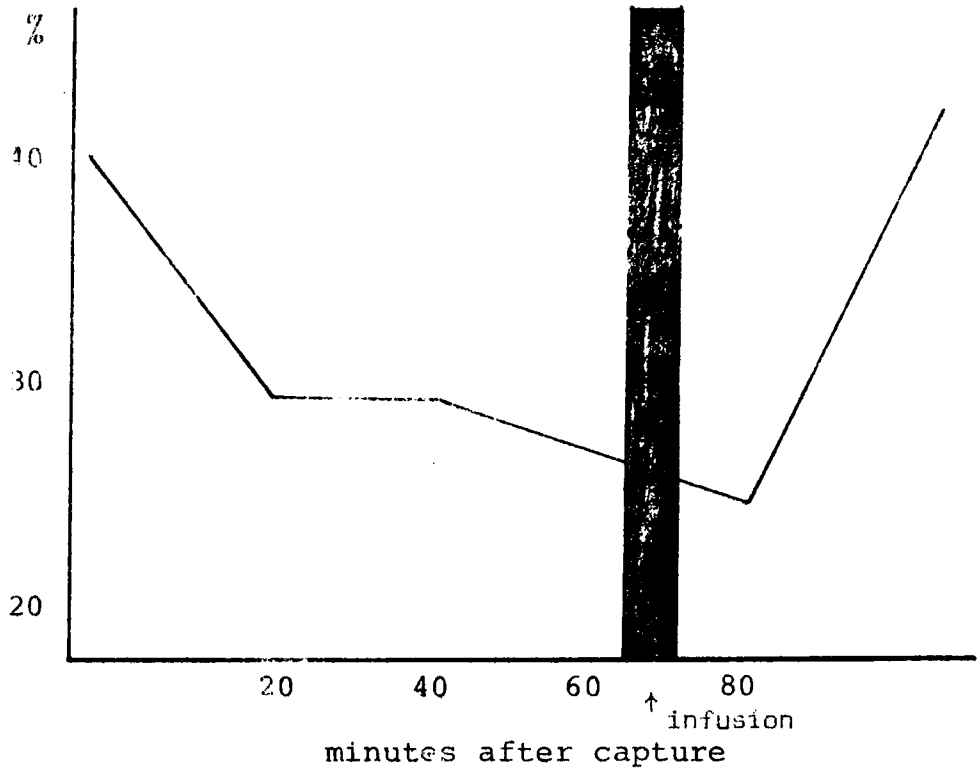


Figure 45: Mean values of haematocrits of three zebra after capture.

elated with restriction in the circulating blood volume.

(e) Low pH, PO_2 and high PCO_2 values in capillary blood samples during the interval prior to infusion as compared to those of venous blood similarly support the concept of sequestrated red blood cells (see also Fig. 29 and 33).

SABLE

SABLE bulls immobilised with fentanyl, xylazine and azaperone showed up to a 32 percent change in the haematocrit values between the first sampling and several hours later (Fig. 46). This change is ascribed mainly to a rise during induction due to splenic contraction following adrenergic

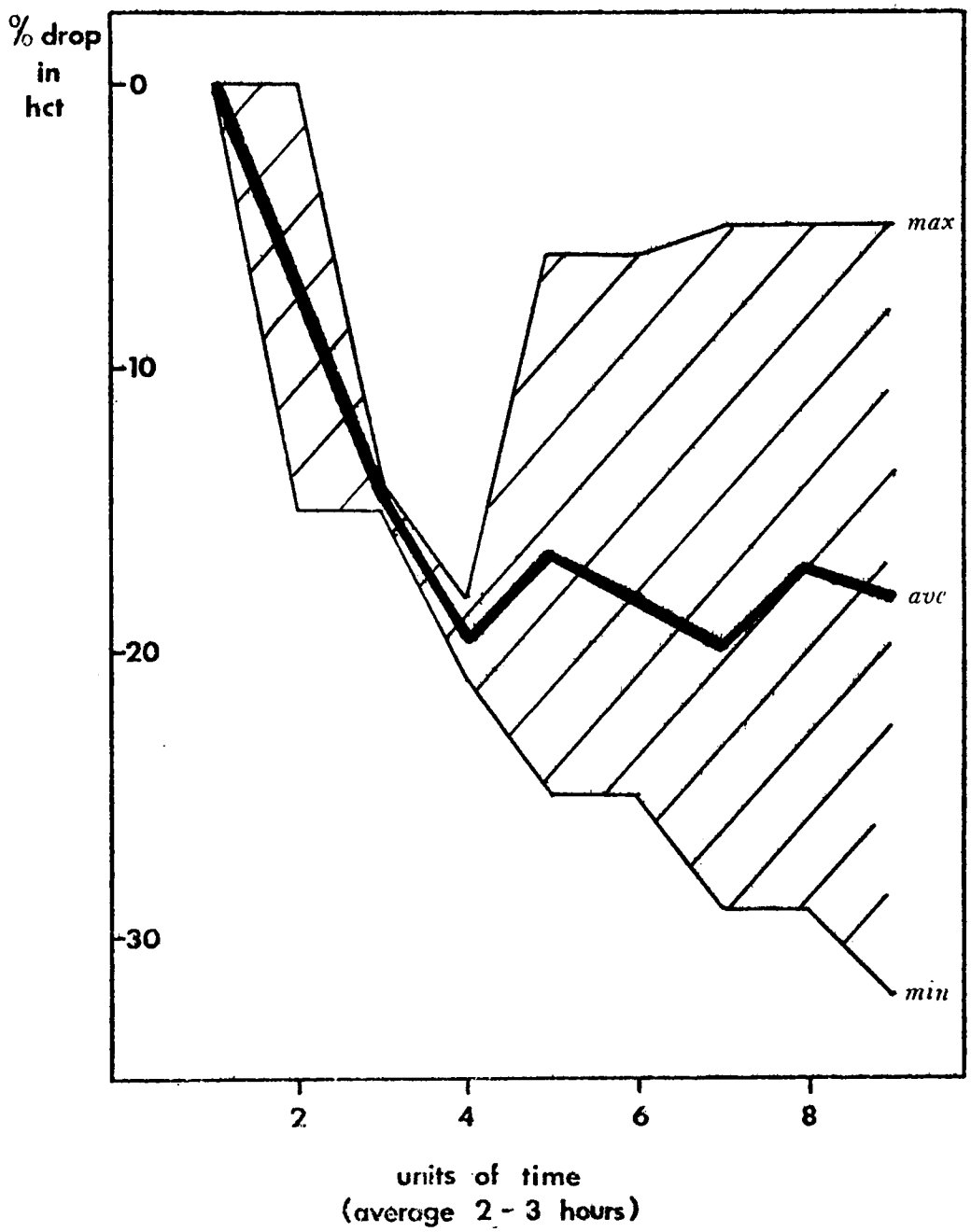


Figure 46: Haematocrit values in nine sable bulls - shaded area bound by extreme values.

discharge. Figure 46 shows a narrow range of fall in haematocrit values in all animals approximately one hour after the first sampling; the subsequent differences are at least in part due to different forms of treatment. The difference at one hour represents 20 percent of the first value. Adrenaline infusion into the immobilised animals did not return the haematocrit value to the original level.

A difference (fall) of 32 percent in the haematocrit value and an average of 20 percent in all animals shortly after immobilisation suggests a considerable error may obtain solely due to the timing of sampling. The plasma protein of these animals remained constant, indicating that no change in the plasma volume or blood dilution accounted for these figures other than the proportion of red cells.

The correlation between the haematocrit values and time after immobilisation was $r = 0,97226^{***}$ $(n = 8)$. The regression line was calculated from the equations $x' = 7,005y - 8,308$ and $y' = 0,135x + 2,04$ and the significance of the steepness of the slope was determined as $t_6 = 11,76129^{***}$.

There is a significant drop in haematocrit values in sable over a period of 200 minutes, although not all the animals were measured over this whole period. Values depicted here are percentages of starting values (Fig. 47).

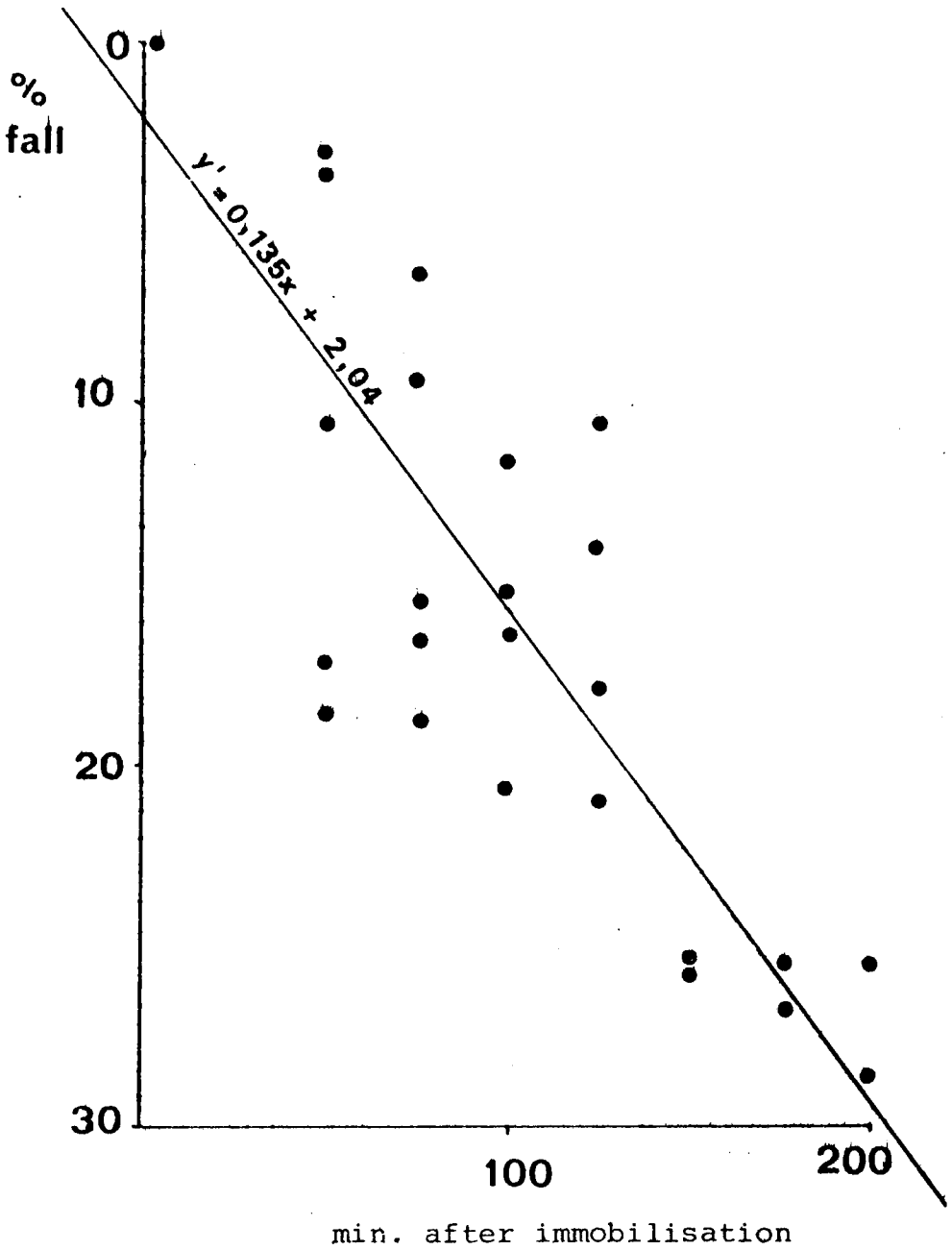


Figure 47: Regression curve plotted over haematocrit values in eight sable antelope after immobilisation.

ELAND

REGULAR sampling of semi-tame eland brought from S.A. Lombard Nature Reserve to Percy Fyfe Nature Reserve provided a valuable indication of the nutritional state (Fig. 48).

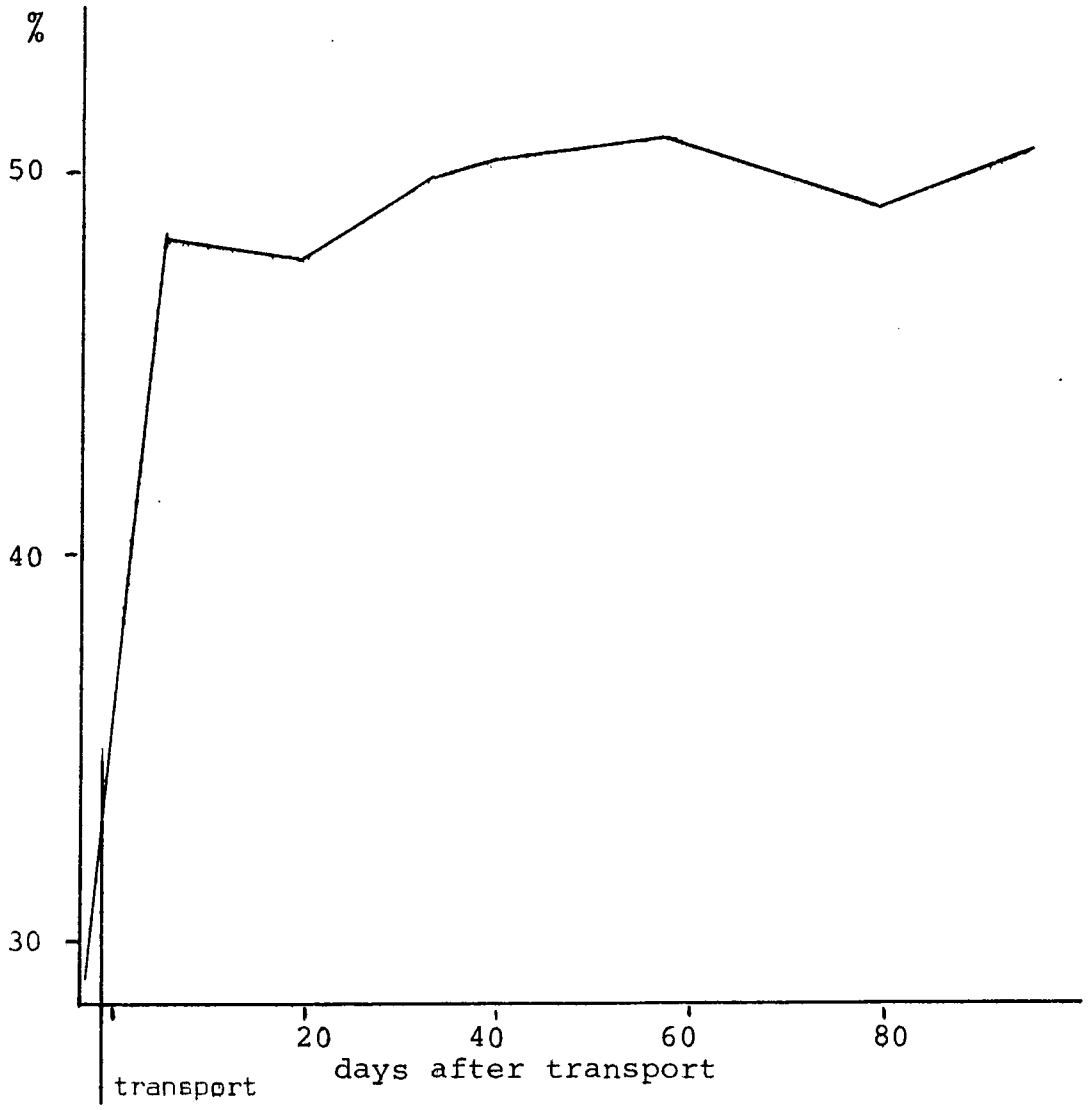


Figure 48: Haematocrit values in eland transported from S.A. Lombard Nature Reserve to Percy Fyfe Nature Reserve (average of 10 animals).

Here, however, an overall fall in the haematocrit values due to the stress of capture with subsequent recovery

cannot be ruled out at this stage. Moreover, a copper deficiency at the S.A. Lombard Nature Reserve has been established since conducting these experiments (Chapter Fourteen).

Because only four measurements were taken before transportation, comparison could only be made of the values before and after transport in four animals. The values before transport were compared to the *average* values over a period of two-and-a-half months because the regression was constant for the Percy Fyfe values (see below). The regression equation is $y' = 0{,}0505x + 46{,}0362$ (Fig. 20). This slope was calculated to be not significantly different from zero ($n = 7$). Using the t-test for paired observations the difference between before and average values after transportation was $t_3 = 4{,}192^{**}$ ($n = 4$), while the Wilcoxon signed rank test on the same results gave T(P,N) = 0, T(N,N) = 10* (note the difference in significance).

There is a significant difference between the values obtained at Percy Fyfe. The values at Percy Fyfe can be regarded as constant, as the regression was not significant. This was also, therefore, a good motivation for using the average of all values after transportation for comparison with the one set of values before transport.

Comparisons were also made of haematocrits before and immediately after exercise, as well as the same values before exercise compared with the peaked values after.

The t-test for paired observations were first made as follows:

before and immediately after: $t_7 = 2,986^{**}$ $(n = 8)$

before and peaked value after: $t_7 = 5,274^{***}$ $(n = 8)$

As a positive correlation appeared to be evident (Table 17), a straightforward correlation coefficient was calculated, which resulted as follows:

before with immediately after: $r = 0,9049^{***}$ $(n = 8)$

before with peaked value after: $r = 0,9323^{***}$ $(n = 8)$

Table 17: Haematocrit values in eland before chase, immediately afterwards and peaked values within thirty minutes.

animal no.→	1	3	4	6	7	8	9	10
before chase (%)	50	48	48	50	56	50	54	48
immed. after (%)	52	49	48	54	56	52	55	49
peak value (%)	53	52	48	54	60	52	56	50

averages: before - 50,5, after - 51,88, peak - 53,13

There was a significant rise in haematocrit values after exercise, with a high positive correlation. Note that the values immediately after exercise were significantly high as well as the peaked values measured within 30 minutes after exercise.

DISCUSSION

HAEMATOCRIT levels in a number of animals have been described here. In zebra there is a considerable fall in the first hour after capture, and similarly in immobilised sable. This compares well with results obtained by Hofmeyr, Louw and du Preez (1973) who showed a fall in haematocrit values in captured zebra. Mountain zebra that were shot and plains zebra immobilised after chasing compared to undisturbed immobilised plains zebra, showed a fall in haematocrit in the chased as well as the standing animals with higher values and a greater fall in the chased animals.

A lead as to the value of haematocrits and their essential stability may be gleaned from work on domestic animals. In horses, changes in the haematocrit may be induced by gentle slapping. The difference between samples taken before and 30 seconds after slapping may be from 44 to 47, i.e. 6 percent (Azzie 1976). Furthermore, there is a difference of 5 to 7 percent in young as compared to old animals and in young females as compared to young males. Moreover, stress conditions may induce an 8 percent difference in the haematocrit in under two weeks, and good feeding an 8 percent increase to a value of over 50 percent (Azzie 1976).

Sporadic tests have been made to check the validity of the haematocrit values. These tests were the following:

(a) Bilirubin tests for cases of rapid destruction of red cells;

(b) haemoglobin estimation, and

(c) mean corpuscular volume, and red blood cell count, and examination of blood smears.

In general, haematocrit values have given useful information only for the following:

(a) Deficiency in trace elements in tamed animals that could be sampled regularly;

(b) capillary haematocrits as indicators of circulatory derangement, and

(c) regular samples in restrained animals, preferably from central blood, to indicate a trend such as progressive haemoconcentration.

Sporadic haematocrits taken from immobilised animals should be interpreted with care, and the same must apply to animals that have been shot, especially when shot through the neck, in which case clinical death follows asphyxiation and massive adrenaline discharge. It is clear that the greatest care must be taken to avoid errors from comparison of tame with wild animals, immobilised with shot animals and so forth.

CHAPTER NINE

MYOGLOBIN AND HAEMOGLOBIN

INTRODUCTION

It was pointed out in Chapter Four that although the highest hydrogen-ion concentrations were found in those animals chased the shortest distance, haemolysis was more evident in those that had covered the largest number of kilometres. Here we have, therefore, two graph lines that cross, namely that of hydrogen ion concentration falling as exhaustion occurs with distance, and that of haemolysis rising. It seems likely, therefore, that the haemolysis factor plays a part in the sub-acute or indefinite phase of capture myopathy.

Analysis of blood has indicated that the discolouration of the blood is in fact not due only to myoglobin as usually surmised, but to a mixture of myoglobin and haemoglobin. We may presume, therefore, that the coffee-coloured urine (referred to as myoglobinuria - Basson and Hofmeyr 1973) is at least to a certain extent a mixture of myoglobin and of haemoglobin derivatives. It seems likely that the urine voided after capture is first red due to myoglobin excretion and later brown due to excretion of degeneration products of haemoglobin pigments. Myoglobin is excreted more rapidly as it has a far smaller molecule than that of haemoglobin

(Molecular weight of myoglobin = 17 000 as compared to 68 000 of haemoglobin), but the difference in excretion is perhaps not important, and difficult to establish due to the problem of obtaining urine from captive wild animals.

ASSOCIATED WORK

DISORDERS INVOLVING MYOGLOBINURIA

HAFF DISEASE

This condition takes its name from the first reported instance which occurred at the Koenigsberg Haff in East Prussia. It is also called myoglobinuria paroxysmalis; about 1 000 cases occurred. The condition is characterised by extreme muscle pain causing the patients to scream at the slightest movement. The muscles are hard, contracted, and respiration is difficult due to pain in the respiratory muscles. The patient is *unable to urinate* at first and when renal function is re-established the urine is of a brownish-blackish colour (Berlin 1948). The urine discolouration was stated to be due to myoglobin, although some red cells were found in the sediment. Haemoglobinaemia was not reported, but the finding of immature red cell forms suggests red cell destruction. The disease ran its course in two to three days.

The mortality rate was only one percent, deaths being due to renal complications following uraemia. The blocking of the renal tubules with myoglobin casts was stated to be the most dreaded complication (Berlin 1948). The condition

was associated with poisoning, and fish, seabirds, foxes and cats were found dead about that time.

A later series of cases occurring in Sweden resulted in two persons dying of 11 affected. The disease was diagnosed primarily in all cases as nephritis. No myoglobin was found in the serum.

'MARCH' HAEMOGLOBINURIA

This occurs as a result of unaccustomed exercise and is stated to be similar to the condition seen in horses under the same circumstances. Characteristic was dark coloured urine and painfully swollen muscles (Stahl 1957). All cases reported were associated with unusual exercise and all recovered.

The cases were compared to paroxysmal haemoglobinuria from chilling, paroxysmal nocturnal haemoglobinuria in chronic haemolytic anaemia, sensitivity to certain plant products (favism), paralytic haemoglobinuria (myoglobinuria) and toxic haemoglobinuria due to severe poisoning or incompatible infusions.

A case of idiopathic myoglobinuria in a negro female was reported (Whisnant Jr., Owings, Cantrell and Cooper 1959). It was noted that the patient was severely oliguric. Potassium intoxication became evident on the second hospital day, and was brought under control by peritoneal dialysis. A normal serum potassium was then maintained with cation

exchange resin. Diuresis only occurred on the 14th hospital day. A renal biopsy performed on the 21st hospital day confirmed the diagnosis of acute tubular necrosis. Characteristic of this condition was stated to be muscle pain, loss of strength, and sometimes paralysis mainly of the legs, but the upper extremities, tongue, jaws and intercostal muscles may be affected. Acute renal insufficiency developed without any obvious cause. When tubular necrosis is present to a degree that a lower nephron syndrome is produced, red blood cells are also found in the urine. It was stated that a positive diagnosis of primary myoglobinuria depends upon the positive identification of pigment in the urine.

It has been pointed out that gross myoglobinuria occurs when large amounts of muscle tissues are destroyed (estimated 200 grams in the adult - Berenbaum, Birch and Moreland 1955). It is suggested that some of the products of muscle breakdown are toxic to the body and creatine, uroporphyrin and haematin should be present, although not in sufficient amounts to cause toxicity.

It was confirmed that deaths from this condition are due to acute uraemia and that the renal lesion responsible is acute tubular necrosis, i.e. lower nephron necrosis (Bywaters and Dible 1943). In a proportion of cases no frank renal insufficiency is noted but renal clearance of para-aminohippuric acid is impaired.

COMPLICATING AND PREDISPOSING FACTORS

IT is clear that renal damage does not always occur in these conditions. Flink (1947) injected large quantities of haemoglobin into dogs (4 to 6 grams per kg) and obtained renal damage only if the plasma concentration rose to above 2,2 grams per 100 ml. Only then did azotaemia, albuminurea and cast formation occur. Haematin, however, when given in doses of 237 mg/kg proved toxic to the tubules.

Whisnant Jr. *et al* (1959) point out that the state of hydration, allergic reactions and increased sympathetic activity which may result in renal vasoconstriction and a reduction in glomerular filtration rate are important. In every case where a marked reduction of filtration rate was produced during injection of haemoglobin or myoglobin, lesions of tubulorrhexis developed (Smith 1951).

The involvement of several differing mechanisms in impairment of urinary excretory function after injury has been confirmed by Bywaters and Popjak (1942). Renal failure tended to follow conditions such as oligaemic shock, haemorrhage, dehydration and electrolyte loss, pericardial tamponade and vascular stasis. The examples given were crushing injury, burns, traumatic liver necrosis, and intravascular haemolysis.

In these types of conditions, blood urea rises rapidly from 40 to 80 mg% and in crushing injury to 400 mg%. A toxic ischaemia was postulated, or oliguria following a

systemic blood pressure insufficient for renal filtration and blood flow.

SURGICAL EVIDENCE

AN indication of the *modus operandi* regarding both the liberation of myoglobin and the effect on the kidney is gained from surgically induced myoglobinuria after acute arterial occlusion (Horowitz, Javid and Zuber 1960). A patient undergoing an operation for a clot in the right iliac artery occurring after a previous operation for resection of the colon, developed myoglobinuria. The myoglobinuria developed shortly after the second operation together with anuria. The GOT was 655 mU/ml, blood urea nitrogen 280 mg/100 ml, potassium 4,5 m-equiv./l. The muscles in the occluded leg showed a loss of striation and of sarcolemmal nuclei with oedema of the muscle fibres. The kidney showed wide-spread tubular necrosis with desquamation of the cells in the distal convoluted tubules and to a greater extent in the proximal convoluted tubules.

A similar case also underwent left-sided iliac embolectomy. The leg showed intense arterial spasm and interstitial oedema. Dark brown urine was voided although the plasma was free of haemolysis. The GOT was 760 mU/ml and blood urea nitrogen did not rise significantly. The leg was removed. Muscle showed patchy areas of ischaemic muscle necrosis with regeneration. Microscopic findings were basophylia of the sarcoplasm, vacuolisation of individual fibres, loss of fibrillary structure, oedema and clumping of the sarcoplasm, marked

sarcolemmal proliferation, focal collections of round cells and haemorrhage. It seems likely that the muscle ischaemia initiates the release of myoglobin from the muscle. Spontaneous myoglobinuria in the horse has been attributed to occlusive disease of the posterior aorta and iliac vessels (Carlström 1955). Experimental arterial occlusion in the dog resulted in release of myoglobin, but only after the circulation had been re-established (Montagnani and Simeone 1953).

The rabbit is stated to have no myoglobin in its muscles, and arterial occlusion resulted in shock, but no renal failure, unless human myoglobin is infused (Bywaters and Popjak 1942, Bywaters and Stead 1944).

Significantly, renal failure could be induced in a rabbit by the injection of purified haemoglobin, but only if the rabbit was producing an acid urine.

Effect Of Unusual Muscle Activity

The use of muscle groups not normally used appears to be conducive to myoglobinuria. A case was reported (Schmid and Mahler 1959) where climbing of steps produced severe and painful muscle cramps followed by transient myoglobinuria, while walking on level ground was tolerated without even undue fatigue. It was also shown that contraction beyond the available nutrient supply of the muscle resulted in cramps, muscle necrosis and myoglobinuria.

Myoglobinuria occurs after fits and particularly after reactions of a violent nature such as electrically-induced spasms. It was noted that the incidence of myoglobinuria from these causes, disappears gradually with increasing body exercise (Ono 1953), thus indicating that it tends to affect muscles that are normally little used, or muscles that are suddenly used to an unusual and excessive degree. A case history of a student who was found to have albumin in the urine, granular and hyaline casts and red blood cells had been forced to rise during the night by fraternity brothers on three occasions and to do 30 to 45 minutes of push-ups and knee-bends (Stahl 1957). Two similar cases exhibiting red-coloured urine had done about 150 knee-bends about 60 hours previously; another subject who had done 200 push-ups similarly produced red-coloured urine.

An interesting series of cases was reported by Howenstine (1960) of recruits who had been made to perform long series of squat jumps which puts strain mainly on the quadriceps muscles. About 60 recruits (0,21%) sought medical care as a result of difficulty in walking and the passing of dark urine, and of these, 12 were hospitalised. The dark urine was passed about 4 hours after exercise and persisted to a maximum of 96 hours in some of the patients. Urine analysis showed myoglobinuria, haemoglobinuria, albuminuria, haematuria, pyuria, granular casts, and red blood cell casts. One recruit continued to exhibit a slight haematuria for seven months. Kidney biopsy showed brown-pigment debris in the tubular lumen and necrosis of the proximal

convoluted tubule. The pathological diagnosis was haemoglobinuric nephrosis. Ultimately these patients were able to perform many squat jumps without discomfort or urinary abnormalities.

The similarities between the overuse of certain muscles during squat jumping and during the repeated jumps that wild animals make while being chased through long grass or into nets (both unusual movements) may be significant.

The exercise need not be excessive to cause the condition. One 19 year old marine performed only 50 squat jumps and within five hours began to observe pain and swelling in both quadriceps muscles. Four days later the patient began to pass mahogany coloured urine which was negative for myoglobin but positive for haemoglobin, and eventually was identified as methaemoglobin and in a similar case the brown pigment was metmyoglobin together with methaemoglobin. Myoglobin appeared to be lost only in the early stages, followed by haemoglobinuria and metmyoglobinuria (Howenstine 1960). It is thought that the escape of red cells from the muscle capillaries and subsequent haemolysis could be the source of the haemoglobin passed in the urine. Another theory was the intravascular haemolysis of red blood cells due to the release of toxic materials from damaged muscle tissue. One substance which has been shown to have a physiological effect which could be deleterious to kidney function is ATP. Others are aldolase, lactic acid, transaminases and haemoglobin. The latter, haemoglobin, has been

shown to have renal vasoconstricting properties (McDonald, Miller and Roach 1951).

MYOGLOBIN IN CAPTURE STRESS

MYOGLOBINURIA is accepted as one of the cardinal signs of capture myopathy. The urine is described as coffee-coloured. As far as may be ascertained from written reports, no tests have actually been made to determine whether this so-called myoglobinuria is indeed a discolouration due to the presence of myoglobin.

CHIEF FACTORS

Discoloured urine is a condition that may arise from several causes and the pigment may derive from differing sources. These may be confused with myoglobinuria and are the following:-

1. CLOUDY URINE

Urine may appear darker when voided. This may be caused by a precipitation of phosphate or carbonate. This tends to occur readily in alkaline urine as the salts are soluble in acid urine. The urine of herbivores tends to be neutral when secreted but readily becomes alkaline due to various causes such as hyperventilation. It also becomes alkaline on standing. Acid urine may be cloudy due to urates. Bacterial growth and leukocytes or pus cells cause cloudiness that may induce alkalinity due to the splitting of urea with consequent formation of ammonia. Mucus is voided in the urine of some herbivores and is increased in inflammatory conditions. Urine may contain prostatic fluid, or spermat-

azoa, but not in a readily visible quantity. Chyluria or fat droplets may cause cloudiness, as may unlysed red blood cells.

2. RED OR REDDISH-BROWN COLOURED URINE

This is due usually to haematuria and the presence of red cells in the blood. If the pigment of the red blood cells is excreted such as through intravascular haemolysis, or if the red cells become subsequently lysed (as readily occurs in hypotonic urine) the urine may be clear red, becoming reddish-brown to brown. If the urine is acid, methaemoglobin may be formed which is of a dark brown or coffee colour. Haematuria may occur as a result of kidney damage, when the clinical symptoms may easily become confused with those of capture myopathy. The secretion of myoglobin into the urine will cause similar discolouration, but brown pigment casts are seen on microscopic examination.

It may be noted that small amounts of haemoglobin pigment released into the blood stream do not appear in the urine, as these are bound by haptoglobin which has haemoglobin-binding properties. The lysis of some 20 ml blood (3 grams haemoglobin) is just sufficient to cause a spill-over into the urine in an animal weighing approximately 75 kg.

Several other causes of dark or red urine are unlikely to be seen in a wild animal, e.g. acute intermittent hepatic porphyria.

3. ORANGE-RED TO ORANGE-BROWN OR YELLOW-BROWN URINE

These types of discolouration occur when the urine contains large amounts of urobilin (urobilogen is colourless but is converted in the presence of light and acid pH to urobilin, which is dark yellow to orange). Yellow-brown to green-brown urine is due to bile pigments such as bilirubin which becomes oxidised to green biliverdin on standing. The bilirubin may be distinguished from urobilin by shaking the urine whereupon a yellow foam appears; normally dark urine or urobilin leaves a white foam.

Melanistic urine occurs in Addison's disease and in malignant melanoma or in alcaptonuria, but these are unlikely to cause confusion in wild animal studies.

The following conditions are recorded in man as causing haemoglobinuria:-

Paroxysmal nocturnal haemoglobinuria

'March' haemoglobinuria, due to unaccustomed exercise

Haff disease

Glucose-6-phosphate dehydrogenase deficiency due to ingestion of certain foodstuffs or medicaments

Cold haemoglobinuria

Auto-immune haemolytic anaemia (Davidsohn and Henry 1969)

In animals, haemoglobin has been found as a result of infections, especially with Babesia causing redwater (Henning 1956).

Myoglobinuria And Plasma Levels Of Haemoglobin And Myoglobin

A number of blood samples from stressed animals have been examined for the presence of myoglobin and for haemoglobin. To distinguish the latter from *in vitro* haemolysis, multiple samples were taken and a colour comparison made. Those that showed a marked increase in colour were then discarded on the assumption that haemolysis due to the sampling technique had occurred.

It is clear from the number of blood samples that have so far been examined, that myoglobinaemia occurs concurrently with haemoglobinaemia, a factor which has, so far, not been postulated. This anomaly may be due to a lack of facilities for the examination of fresh blood and urine, but also, on account of the reliance on urine examination alone.

The mechanisms of liberation of myoglobin through degeneration of the sarcolemma of muscle fibres is thought to be understood (Chapter One, *inter alia*, on muscle degeneration due to pH). It should be noted that the recovered myoglobin represents extensive muscle damage, on the basis that myoglobin constitutes (on a dry rate basis) only two to three percent of skeletal muscle.

It appears that haemoglobin is also liberated in substantial quantities during intensive muscular exercise, possible ischaemic muscular exercise and the exposure of muscle fibres to excessively low pH. Some may be derived from intravascular haemolysis, possibly from jarring as animals

are chased over hard ground in winter. This haemoglobin is not always found in the urine and therefore not readily estimated by urine analysis due to its larger molecular weight and immobilisation in the blood serum by haptoglobin as explained below.

Selective Binding Of Haemoglobin

Haptoglobin was first demonstrated and named by Polonovsky and Jayle (1939). This factor selectively binds haemoglobin and, virtually the entire haemoglobin-binding capacity of serum is thought to be due to its haptoglobin content (Javid, Fischer and Spaet 1959). The quantity of haptoglobin in 100 ml serum is able to bind approximately 125 mg haemoglobin (Allison and ap Rees 1957). The excretion of haemoglobin does not commence until the haemoglobin-binding capacity of the blood is exceeded (Allison and ap Rees 1957, Laurell and Nyman 1957). Thus there is an apparently 'high threshhold' for the excretion of haemoglobin. On the other hand, myoglobin is passed readily into the urine, even low plasma concentrations of 15 mg/100 ml blood (15 mg%) as compared to 125 mg% for haemoglobin.

The molecular weight of the two substances differs considerably. That of haemoglobin is 68 000 (i.e. similar to that of serum albumin which does not normally pass into the urine except in very small amounts), while that of myoglobin is only 17 000. Furthermore, the binding of haemoglobin to haptoglobin increases the molecular weight to approximately 210 000 (Laurell and Nyman 1957).

It may be noted, however, that although the 'renal threshhold' is 125 to 135 mg% it may be lowered by 60% through daily injection of haemoglobin (Yuile, Steinman, Hahn and Clark 1941), causing the haptoglobin to become inactivated; although there is possibly some tubular reabsorption.

MATERIALS AND METHODS

A series of blood samples was taken from 18 blesbok which had been pursued for various distances up to 10 km. The method of analysis is described in Chapter Two.

RESULTS

Quantity Of Total Porphins Present In The Blood Of Stressed Animals

The term porphin is used for the 4-pyrole nuclei joined together by CH groupings. Derived from this parent substance is porphyrin. The porphyrins are characterised by different substituent groupings in positions one to eight in the porphin ring system. The urine and faeces normally contain 100 to 300 µg porphyrins per day and these are derived from ingested material as byproducts of haemoglobin and myoglobin formation. Increased elimination of porphyrins occurs in diseases of the liver. Chlorophyll is a magnesium porphyrin (Bell, Davidson and Scarborough 1961).

The results show a steep rise of pigments as the distance of the chase is prolonged. Those pursued for only short distances show no presence of abnormal pigments (Fig. 49).

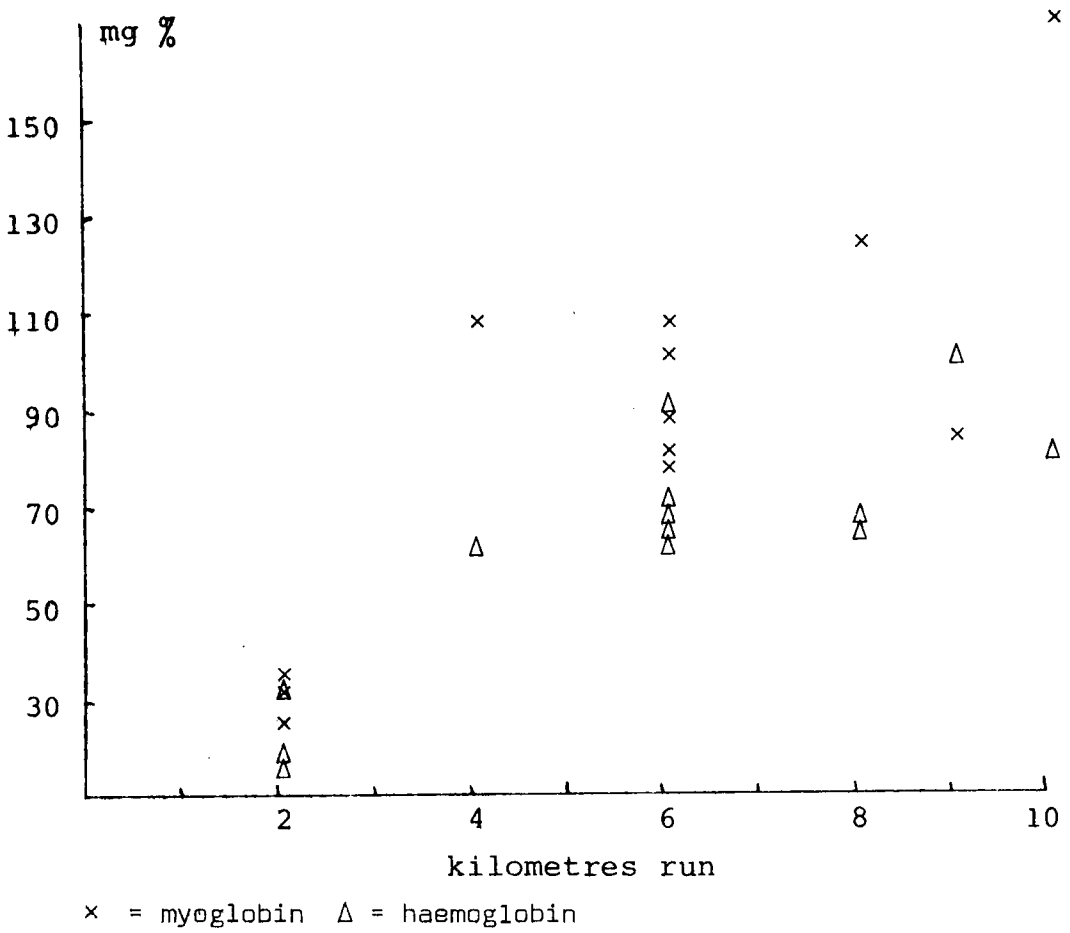


Figure 49: Myoglobin and haemoglobin levels found in plasma of blesbok after chasing over distances of two to ten kilometres.

A correlation was calculated for both haemoglobin and myoglobin with distance run. The resulting coefficients were as follows:

Haemoglobin: $r_{xy} = 0,8080^{***}$

Myoglobin: $r_{xy} = 0,7850^{***}$

DISCUSSION

THE correlation of myoglobin and haemoglobin with distance run is highly significant. The high positive correlation indicates that the further the distance, the greater the concentration of haemoglobin and myoglobin in the blood serum, which acounts for the greater degree of discolouration of the serum after longer runs.

The significance of this is not fully understood but it seems likely that even during capture operations, it may follow this pattern:

(a) Sharp fall in pH; (established)

(b) fall in systemic blood pressure; (established)

(c) fall in perfusion pressure;

(d) lowered muscle pH as compared to blood pH; (established)

(e) degeneration of muscle tissue due to excessively low pH values causing myoglobin to pass into the circulation;

(f) regional low PO_2 values and high pH levels preventing regeneration of ATP;

(g) muscles becoming rigid on a local basis and muscle fibres rupturing during the intensive exercise under these circumstances;

(h) local haemorrhages resulting in the passage of the red blood cells into the tissues followed by the release of haemoglobin into the circulation, and

(i) the trauma to the body of running on hard ground in the winter causing breakdown of red blood cells.†

† Athletes may show haemoglobinuria after prolonged running on a hard surface and none on soft ground (Davidson 1964, Buckle 1965).

Examination of kidney materials taken on autopsy from animals that have died from capture stress shows tubular necrosis and casts of material that may be derived from haemoglobin or from myoglobin. Traditional experiments indicate that substantial amounts of blood and muscle pigments may be injected with little risk of kidney damage and death from renal failure does not occur in any but a low proportion of cases of myoglobinuria occurring from various pathological causes such as acute poisoning (see Haff disease above).

This does not mean that renal failure may not be induced by the substances under the condition of capture stress and the resultant captivity. Any additional factors such as reduced blood flow through the kidney may render that organ very much more susceptible to blockage of the tubular structure. Other factors that may contribute are tubular degeneration and necrosis, and a lowered urinary output.

Lowered urinary flow is bound to follow from:

(a) Reduced systemic pressure;

(b) reduced regional perfusion pressure;

(c) renal vasoconstriction due to sympathetic discharge;

(d) renal vasoconstriction due to fall in pH and to the effect of haemoglobin (McDonald, Miller and Roach 1951);

(The latter two (c) and (d) have been established by experimental procedures in dogs and are accepted as physiopathological mechanisms - Guyton 1966)

(e) dehydration and reduced urinary output;

(f) anoxaemic degeneration;

(g) continued adrenergic hypertonus, and excessive lactate formation on a long term basis during captivity.

It is believed that the release of myoglobin and haemoglobin into the blood plasma may be reduced by improved methods of capture (see Chapter Seventeen). It is expected that continuous high sympathetic tonus and failure to take water (and food) after capture may be remedied by changes in the holding and quarantine enclosures from the present highly artificial system of solitary confinement in slatted stalls.

The infusion of fluid, perferrably of an alkalising nature should theoretically be valuable but probably rarely practicable. The possibility of inducing pre-capture intake of anxiolytics is discussed in Chapter Seventeen. This would reduce sympathetic tonus, promote food and water intake, and thus provide a vehicle for medication.

CHAPTER TEN

ENZYMES

INTRODUCTION

DURING exploratory work changes in a number of blood enzymes have been investigated including sorbitol dehydrogenase, isocitrate dehydrogenase, maleate dehydrogenase and phosphoglucose isomerase. Eventually the range was narrowed down to four enzymes which most closely reflected the various aspects of capture stress. These enzymes are the following:-

Glutamic oxaloacetic transaminase (GOT)

Glutamic pyruvic transaminase (GPT)

Creatine phosphokinase (CPK)

Lactate Dehydrogenase (LDH)

Serum enzymology provides aid in making the diagnosis, monitoring the course, and demonstrating subclinical evidence of those conditions that are characterised by distinctly abnormal values of one or more enzymes. The diagnostic circumstances that are most clearly aided by serum enzymology are the differential diagnosis between conditions such as myocardial damage and of hepatobiliary and muscle disease.

These enzymes indicate pathological processes and aberrations in various organs and tissues: e.g. Hepatic necrosis leads to high values for GOT and GPT but normal values for

CPK; skeletal muscular dystrophy (in man) leads to striking elevations of CPK, moderate levels of LDH values, slight elevations of the GOT level and lesser values of GPT (Davidsohn and Henry 1969). The diagnostic application of serum enzyme assays is based on clinical experience mainly in man and on experimental data. Thus the factors are formulated that lead to abnormal enzyme values and correlation of serum enzyme values with the nature of the pathological process and the organ or organs involved.

MATERIALS AND METHODS

ENZYME work has been performed on eight species, namely, blesbok, tsessebe, zebra, wildebeest, nyala, white rhinoceros, sable antelope and eland. The work on nyala was a brief investigation into two deaths and is described in Appendix C. Enzyme levels in rhinoceros were found to be apparently normal and are described in Chapter Three. Only sporadic values were obtained in sable antelope and are not described here. Enzyme levels in tsessebeto determine effectiveness of therapeutic substances are discussed in Chapter Fifteen. Eland, zebra, wildebeest and blesbok have been subjected to serial tests and are discussed in this chapter. Methods used in the determination of enzyme levels are described in Chapter Two.

RESULTS

ELAND

A comparison was made between the effects of stress on enzyme levels after transportation and after forced exercise around a track after an interval of four-and-a-half months' resting period.

The difference between the values before transport and the peaks after transport were calculated (Table 18) and compared with the differences between values before and the peaks after exercise. The t-test for paired observations was used for this comparison and the results were as follows (n = 8):

GOT : t_7 = 2,616**

GPT : t_7 = 1,521 not significant

CPK : t_7 = 18,789***

LDH : t_7 = 2,083*

There are significant differences in stress levels reflected by three enzymes between transport and exercise. GPT, however, reflects no statistically significant difference between the two types of stress. In GOT,GPT and CPK the levels are much higher after exercise than after transport, but the LDH reflects a higher degree of stress after transport than after exercise.

Table 18: Comparison between enzyme levels after transport and enzyme levels after exercise.

	range of peak after transport (mU/ml)				range of peak after exercise (mU/ml)			
	GOT	GPT	CPK	LDH	GOT	GPT	CPK	LDH
animal no.								
1	14,0	15	7,5	1277	137	62	716	274
3	3,5	5	9	539	96	96	840	52
4	1,5	-5	10	359	67	9	893	52
6	48	5,5	-4	344	172	12	899	197
7	219,5	42,5	7	435	127	26	813	50
8	9,0	33	5	129	60	9	912	44
9	-13,5	6	-2,5	458	45	3	554	122
10	10,0	10	-22,5	201	97	66	727	608
average	36,5	14	1,19	468	100	35	794	175

Other enzyme work was performed on eland in relation to establishment of resting values, and this is discussed in Chapter Three. Further work on enzymes in eland to reflect effectiveness of hydrocortisone therapy is discussed in Chapter Fifteen.

ZEBRA

ALL zebras subjected to locomotory stress showed high blood enzyme levels. All enzymes rose after capture in contrast to wildebeest which showed apparent peak levels approximately at the time they were caught.

The average levels of LDH rose to 2 750 mU/ml in about five days after capture. With regard to the interval after capture, it should be noted that wildebeest and zebras were sampled twice weekly only. More frequent sampling would have induced undue extra stress; even twice weekly tended to induce damage as the animals were roped and cast.

Return to normal values was, however, rapid and all animals showed a considerable drop in LDH values by the tenth day. Surprisingly the second chase induced only a minor rise in the LDH levels to 750 mU/ml. The reason for this is not definitely known at this stage. It was clear, however, that the animals did not exert themselves unduly at the time of the second chase (Fig. 50), either because of a taming aspect or else from muscle stiffness resulting from a month in the pens. The result fits in, however, with the general findings on repeat runs and the effect of previous exercise or training (Chapter Fifteen).

Presumed normal levels of LDH were attained about sixty days after the first chase or thirty days after the second chase (Fig. 19). Normal levels were taken as those at which the curves flattened consistently. These levels were close to those reported for the horse, i.e. a maximum of 375 mU/ml (Chapter Three). The levels are on the high side of normal ranges presumably due to stress of captivity and the semi-weekly sampling. The rise in levels of CPK showed a similar pattern to the LDH (Fig. 50). The average for all zebras was 660 mU/ml, falling again in ten days

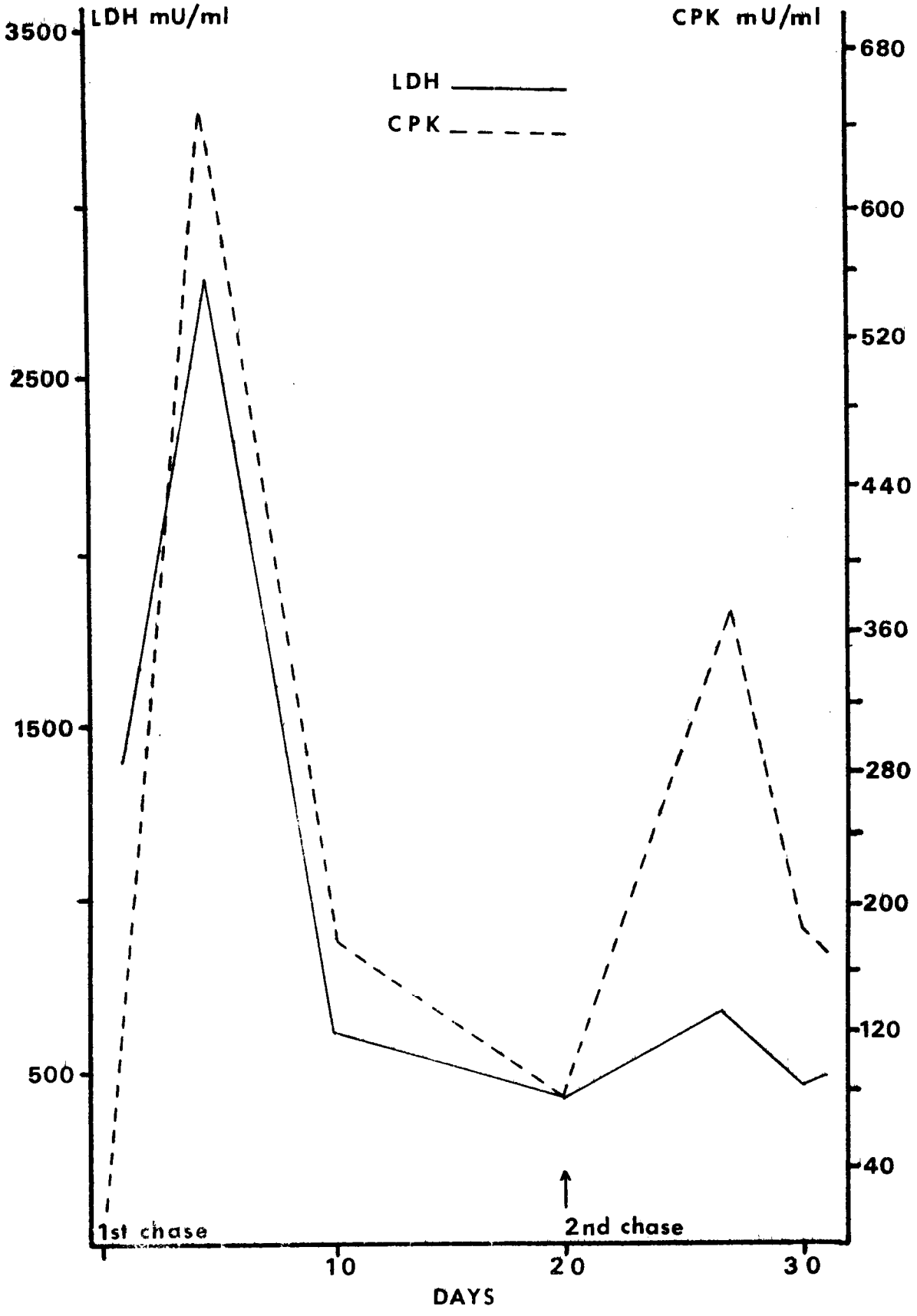


Figure 50: LDH and CPK levels in zebra after maximum forced exercise in the Kruger National Park.

after chasing to 160 mU/ml and then to 80 mU/ml in twenty days.

At the time of the second chase the levels of CPK rose proportionately much higher than did those of LDH, although the average value, 360 mU/ml is only just over half of that of the earlier level. Ten days after the first and second chases, the levels were almost identical at 160 and 179 mU/ml respectively, and near normal levels were achieved on the fiftieth day after chasing.

Levels of GPT in zebra showed very similar changes as did the other two enzymes mentioned above. The main difference was that they still showed normal values on capture, but rose rapidly to 165 mU/ml in approximately five days' time with an equally rapid return to normal values at ten days after exercise.

The relatively high levels of CPK after the second chase as compared to LDH may be due to differential strain on the relevant organs. The skeletal musculature was used to carry the animals' weight over the requisite distance irrespective of speed or circumstances. On the other hand since LDH seems to be associated with shock and anoxia, lower levels would be consistent with a smaller degree of exertion or possibly reduced fear due to a degree of taming or training.

There was a small rise of GPT as a result of the second

chase when levels rose only to 23 mU/ml. Again, this difference in levels between the first and the second chase is difficult to explain with certainty. GPT is the principal enzyme stated to indicate hepatobiliary disease. It does rise in heart failure and shock, however, especially if there is attendant biliary necrosis. *Post mortem* examination of deaths from capture myopathy show lesions of hepatic necrosis. This condition is unlikely to occur as a result of effort that does not lead to actual circulatory derangement. If these results are reproducable they may indicate different degrees of involvement of various organs in relation to the speed, length and conditions of the exercise.

The changes in GOT levels differ from those of the other enzymes largely in that the rise at the time of the second chase was much higher than after the first, i.e. 250 as compared to 170 mU/ml (Fig. 51). This may be explained possibly on the basis of an organ such as the heart being stressed while still in a convalescent state from previous exertion, i.e. with unresolved lesions in the myocardium resulting from the previous forced exercise. The enzyme GOT is found in a wide spectrum of organs so that the origin of high GOT levels at the time of the second chase is difficult to place exactly. All treated animals survived both bouts of exercise, so that it was not possible to correlate enzyme levels with lesions manifested at autopsy. All untreated animals died within 12 hours, at which time enzyme levels had just commenced to rise and specific org-

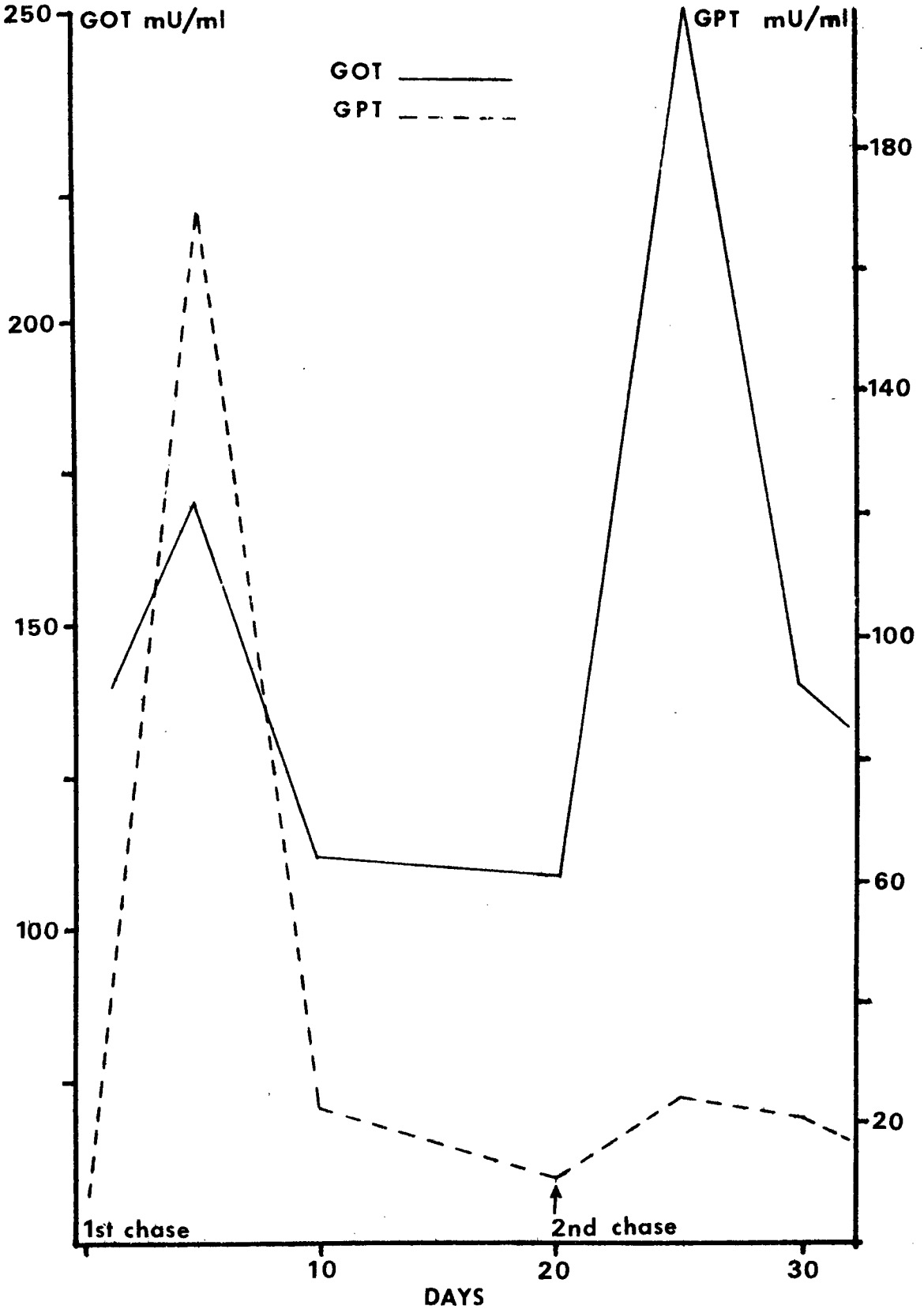


Figure 51: GOT and GPT levels in zebra after maximum forced exercise in the Kruger National Park.

an lesions were undetectable within the general picture of acute death from palmonary oedema.

Blue Wildebeest

The enzyme levels in blue wildebeest showed similar curves to that of zebra. The wildebeest, however, were chased only once.

With the exception of GOT, enzyme levels reached peak values earlier in the wildebeest than in the zebra. As a result the levels did not continue to rise after capture. LDH levels reached an average of 1 289 mU/ml at capture as compared to 1 400 mU/ml in zebra, but showed no further rise. On the other hand the fall to normal levels was slower so that these were attained only fully two months later, the fall being gradual (Fig. 52).

Levels of CPK averaged 299 mU/ml on capture as compared to only 40 for zebra. The zebra, however, rose rapidly to 640 mU/ml while the wildebeest showed a gradual fall from the capture sample to normal levels a month later.

Levels of GPT are unremarkable. Again a maximum rise was evident immediately after capture. This enzyme did not rise to any high levels, but took two months to return to what are presumably normal values. In blesbok, levels returned to normal in less than two weeks or about a quarter of this time.

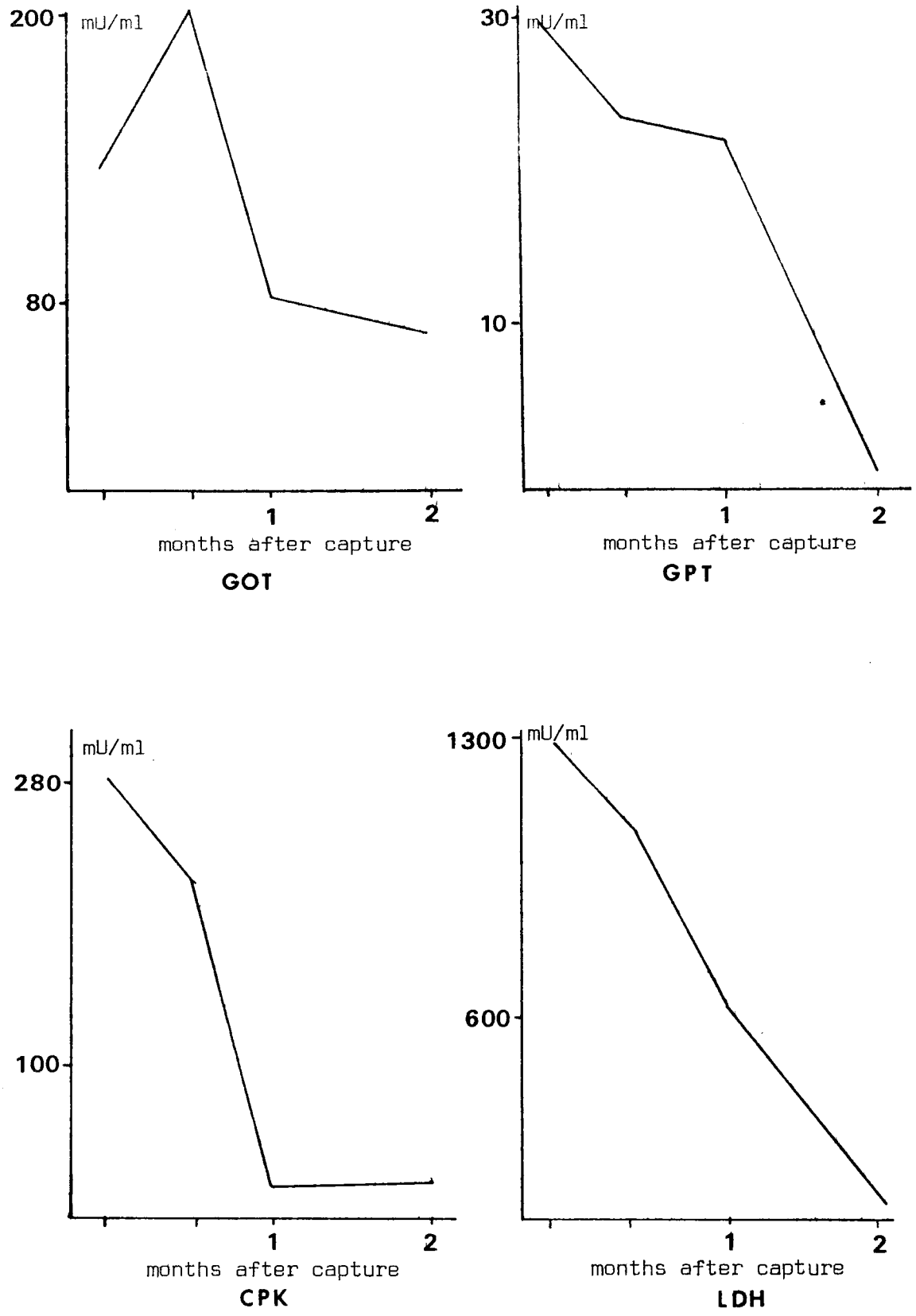


Figure 52: Enzyme levels in blue wildebeest after maximum forced exercise in the Kruger National Park.

If indeed this enzyme reflects hepatobiliary conditions in wildebeest as it is stated to do in man, it would suggest low grade hepatic damage that either takes a long time to resolve or else continues to manifest itself as a result of captive conditions, repeated sampling or artificial foods.

In wildebeest the levels of GOT showed a rise over the first two weeks which is different from findings in zebra and blesbok. The fall to normal levels again took two months although there was a sharp fall between the second and third weeks. Normal levels are still uncertain for this species as the curve did not flatten fully over the time of investigation. This enzyme shows considerable differences amongst the various species examined. In man the normal level is stated to be 12 mU/ml, but in the horse it is stated to be 40 to 100 mU/ml (Chapter Three).

BLESBOK

ALL enzymes reflected a rise after exercise, GOT, GPT and CPK peaking after three days. LDH, however, continued to rise up to eight days after exercise. GPT and CPK levels returned to normal levels within nine days, with GOT and LDH gradually returning to normal, taking slightly longer to recover than the GPT and CPK values.

Acute Enzyme Rises

EXAMINATION of enzyme levels has also been carried out on a short-term basis concentrating on multiple samples being taken from the time immediately after capture at 30 minute intervals until the time of release some 60 or 90 minutes later.

As a result a slightly different picture was obtained to that described above, mainly in respect of wildebeest. The previous enzyme curves indicated that there was an immediate drop in all values from the time of capture gradually attaining normal levels over the ensuing month or so after capture. Multiple samples taken, starting immediately after capture indicated that there was a rise in enzyme levels in wildebeest of GOT, GPT and CPK but a fall in LDH. Zebra presented much the same type of picture with steep rises in CPK levels. Here the LDH likewise showed a rise during the 90 minute period after capture, while the results of GOT and GPT were indeterminate (Fig. 53 and 54).

DISCUSSION

CONSIDERABLY more work will have to be carried out before blood enzymes in wild animals can be used for clinical diagnostic procedures to reflect a clear picture of organ damage.

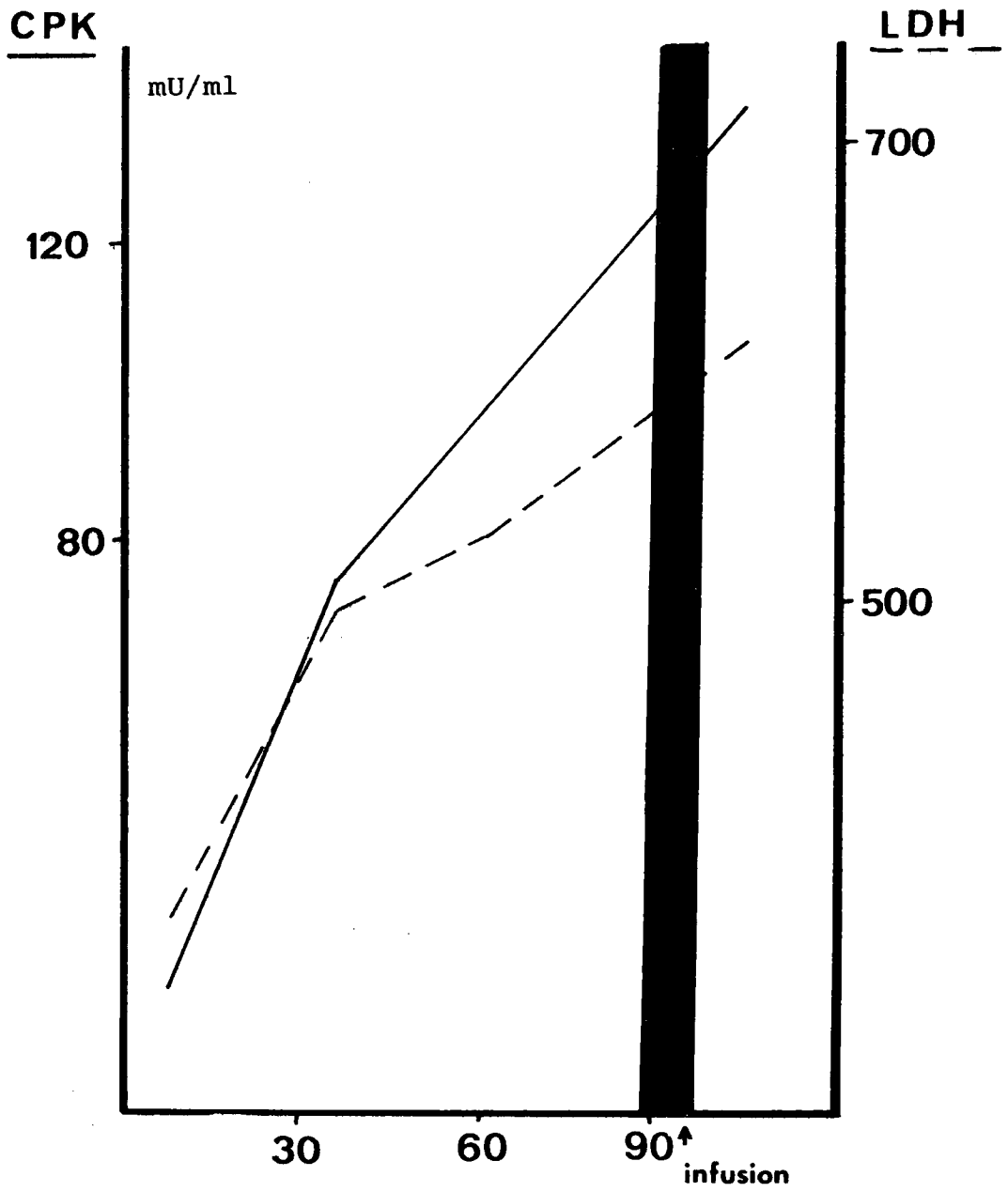
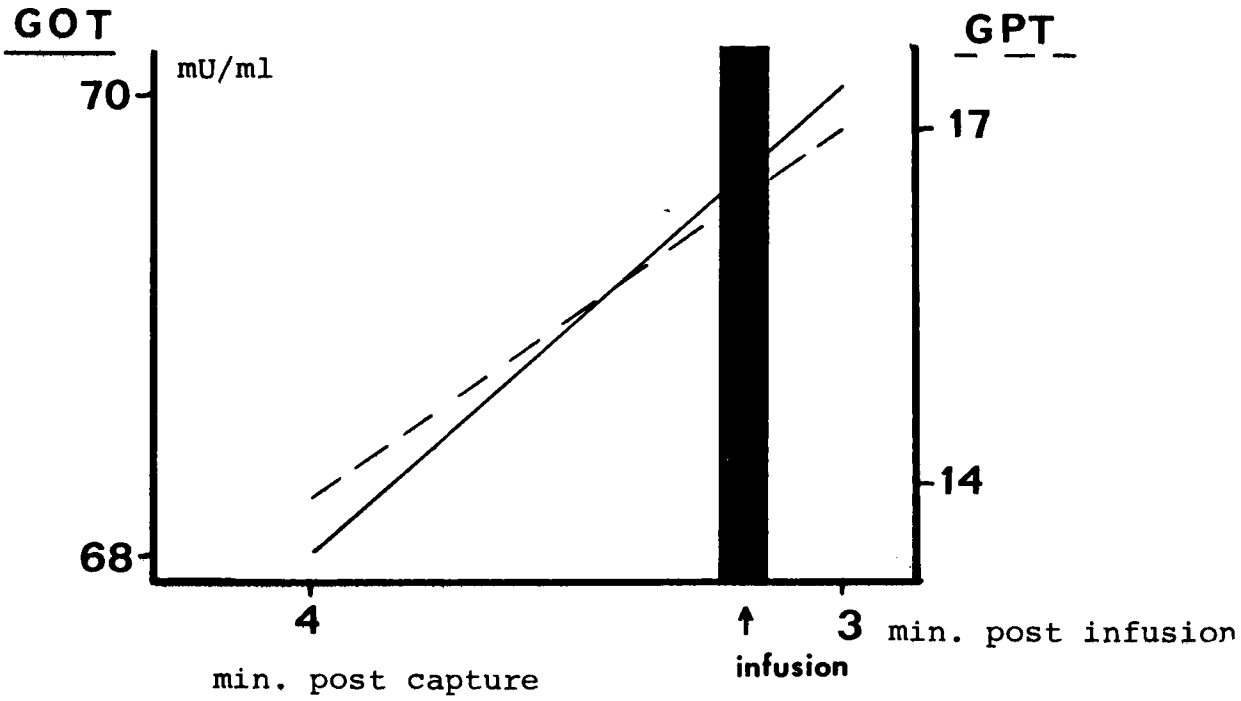


Figure 53: CPK and LDH levels in zebra after capture and after bicarbonate infusion therapy.



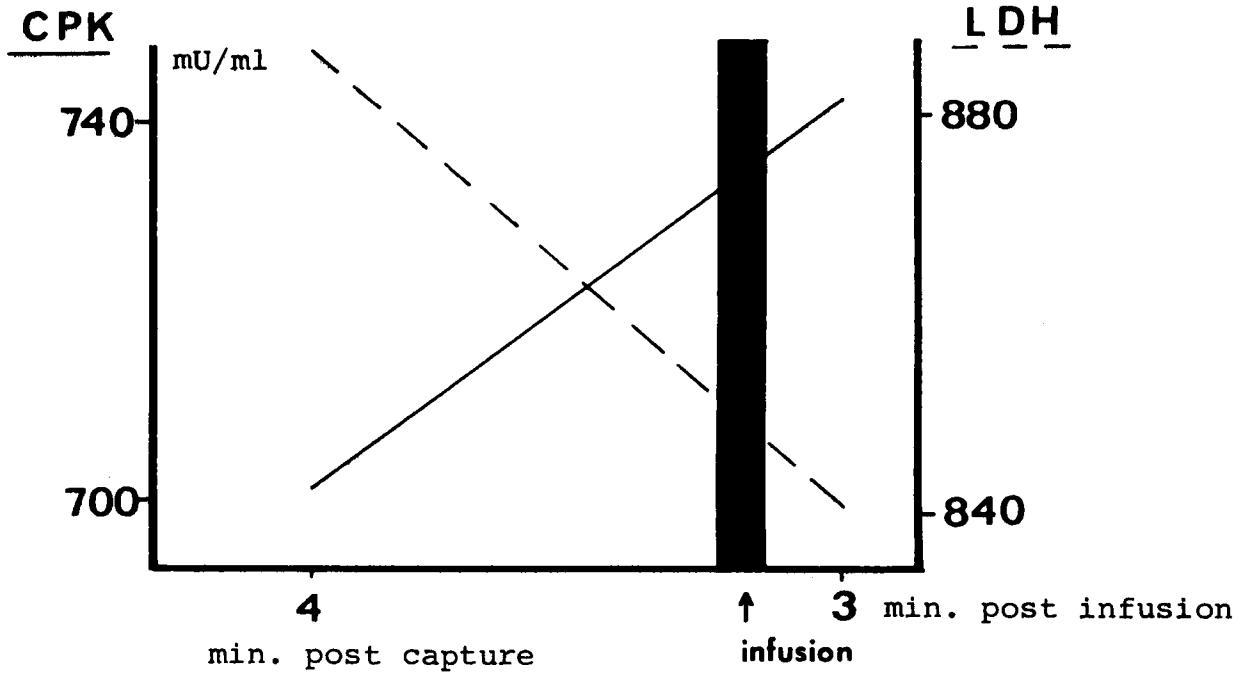


Figure 54: Enzyme levels in blue wildebeest immediately after capture and after bicarbonate infusion therapy.

To date, enzyme studies have been carried out on only eight species. The results of four species have been described above. Further results of the remaining species are discussed in Chapter Fifteen. The enzyme estimations on tsessebe described in Chapter Fifteen were performed after forced exercise under specialised conditions on a track at Percy Fyfe Nature Reserve and the value of comparison with free running blesbok, or wildebeest and zebra in the Kruger National Park is uncertain. On the other hand, free living animals do not react as do trained domestic animals and the rise in CPK in zebra reflects that of untrained rather than that of trained horses (Riethmüller and Wels 1972).

Enzyme levels in the test zebra showed greater rises than those reported by Hofmeyr, Louw and du Preez (1973), but samples in the experiments of these authors were taken only up till one hour after immobilisation and thus the full rise was probably not determined.

The fact that enzyme levels do rise, apparently consistently with degrees of locomotory stress and exercise, suggests that they form a valuable indication as to the degree of stress and resultant bodily changes. It is probable that changes in enzyme levels may also give useful and accurate indications of the effectiveness of therapy, and to times after capture, when animals can be moved.

High plasma enzyme levels clearly may reflect non-lethal

conditions. The high levels seen in the wildebeest and in the zebra treated with bicarbonate reflected damage or shock from which these animals recovered. Very high levels of enzymes such as lactate dehydrogenase were also reported by Gericke and Hofmeyr (1976) for springbok, but it was not mentioned whether these animals survived. It would appear that enzyme levels reflect stresses that are, as in the zebra and wildebeest mentioned above not always lethal. Low pH values in zebra, or high potassium levels in sable, on the other hand, did prove lethal and thus may be regarded as a more serious indicator of impending capture myopathy. It seems highly likely, however, that further stress inflicted on animals while the enzymes are at a peak or still at high levels, may precipitate a fatal stress condition. This may account for the unexpectedly high number of deaths in animals such as springbok moved about one week after capture when in superficially reasonably good condition (own records).

CHAPTER ELEVEN

BLOOD METABOLITES

INTRODUCTION

METABOLITES are formed in the blood as waste products and are removed by the kidney to be excreted as urine. When there is depression of kidney function, the proportion of these substances in the blood will rise above normal levels. The condition of kidneys of animals that died soon after capture gave indication that renal failure was implicated as a cause of death.

Renal failure may be due to a number of factors. Such factors occurring under capture conditions are:-

(a) Blockage of renal tubules by myoglobin. Although both myoglobin and haemoglobin are released into the blood stream, the former has a smaller molecular weight, and is therefore excreted more readily (Chapter Nine). It is therefore more likely to precipitate in the renal tubules.

(b) Excessive acidity has been shown to cause spasm of pulmonary blood vessels (Chapter Six). The effect on the kidney vessels is not known. It is certain, however, that acid conditions promote the precipitation of pigments in the renal tubule as metmyoglobin and methaemoglobin.

(c) A low systemic blood pressure will induce a proportionately far greater fall in kidney perfusion

pressure. Below 100 mm Hg, there is very little, if any, kidney blood flow. The fall in systemic pressure to this level together with a rise in the pulmonary artery pressure, appears to be a common phenomenon after intensive exercise and resultant acidaemia (Chapter Six).

(d) Adrenergic discharge in wild animals is high, and tends to be continuous under capture conditions, particularly in the early stages. Even low levels of adrenaline appear to cause renal vasospasm (see discussion below).

MATERIALS AND METHODS

EIGHT eland and six sable antelope were used in this series of experiments. Eland were subjected to forced exercise on an exercise track and the sable were immobilised and given a slow adrenaline infusion in some cases mixed with phenoxybenzamine hydrochloride given at a rate of 10 mg per litre over one hour. Details of these procedures as well as details of analysis of creatinine, blood urea nitrogen and urea are described in Chapter Two.

RESULTS

ELAND

CREATININE

FIGURE 55 shows the values of creatinine after exercise.

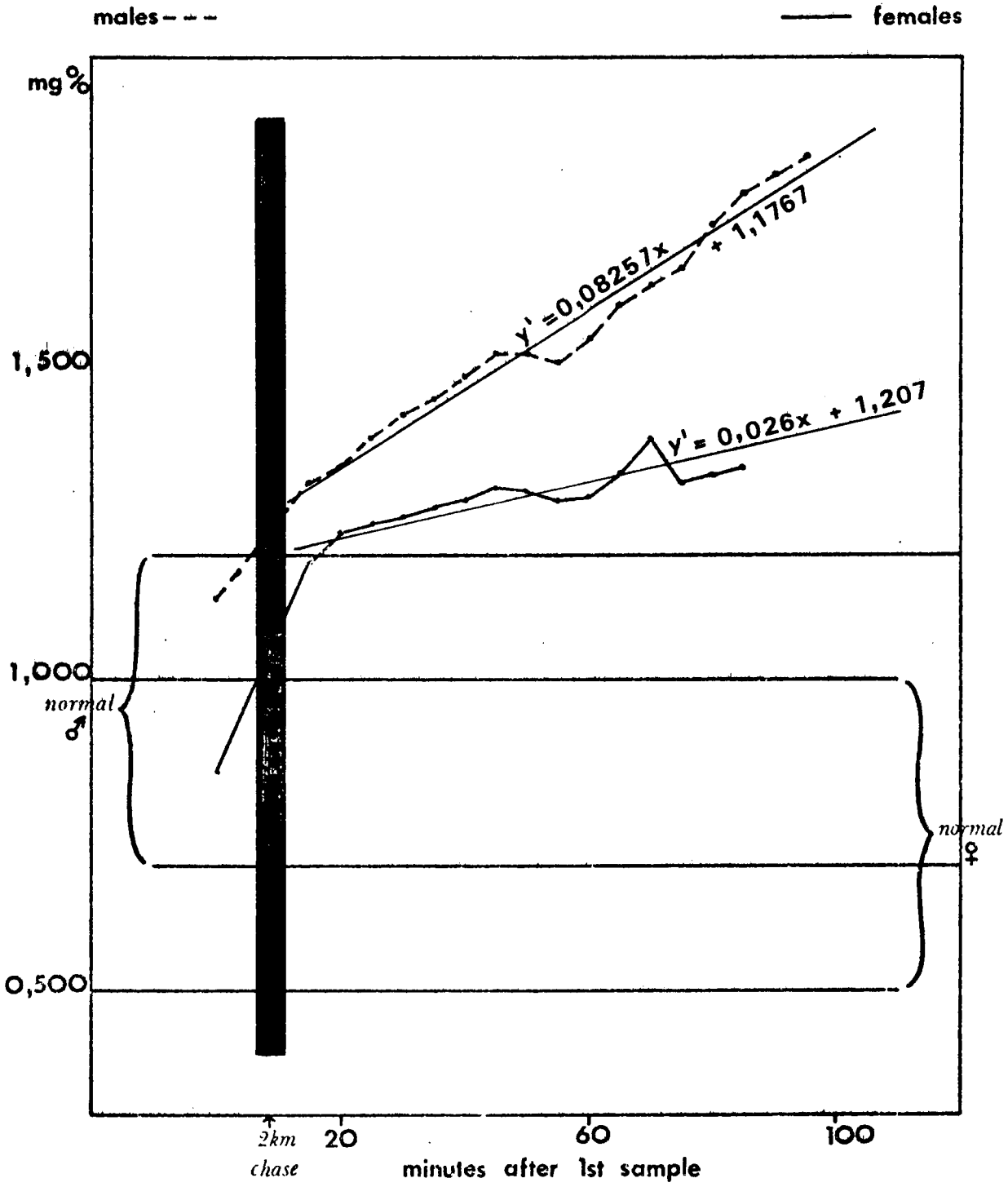


Figure 55: Creatinine levels in four female and four male eland before and after exercise.

The mean values show a steep rise over the period of restraint, although very little struggling occurred. These results may be due either to increased formation of these substances or to a failure or depression of excretion. Whereas creatinine especially tends to rise as a result of muscle action, there appears to be only a small rise in creatinine level as a result of the 2 km run, the main rise of creatinine coming towards the end of the sample period at some 80 to 100 minutes after first sampling just prior to exercise.

The *t*-test for paired observations was applied to the difference in males and females between *before* exercise values and immediately *after* exercise values. The results were as follows:

males: $t_3 = 3{,}298^{**}$
females: $t_3 = 3{,}463^{**}$ } $(n = 4)$

The Wilcoxon signed rank test was used because the sample size was small and the same results were obtained:

T(P,N) = 0, T(N,N) = 10^{**} for males

T(P,N) = 0, T(N,N) = 10^{**} for females

Males were also compared with *females* both before and after chase. The results were as follows:

before chase difference : $t_6 = 1{,}6206^{*}$ (one-sided)

after chase difference : $t_6 = 0{,}8604$ not significant

The regression lines for males and females were calculated and the significance of their different slopes obtained as

follows: (one-sided):

males $r_{xy} = 0{\cdot}934^{***}$
$y' = 0{,}08257x + 1{,}1767$ } $(n = 6)$
$t = 6{,}0^{***}$

females $r_{xy} = 0{,}9165^{***}$ (actually, 2%)
$y' = 0{,}026x + 1{,}207$ } $(n = 6)$
$t = 5{,}619^{***}$

To establish a significant difference between the slope of the values for females and the slope of the male values, the value $t_{10} = 2{,}366^{**}$ was obtained.

Three main sets of observations were analysed here.

(a) The difference between values before exercise and values after exercise were proved statistically significant regardless of sex.

(b) Immediately after exercise, the values of males and females were compared. Whereas before exercise, there was a significant difference between males and females (this was expected and was therefore a one-sided problem), after exercise there was no significant difference, indicating both were stressed to a similar degree.

(c) The rate of rise in creatinine levels was measured in males and females separately. Both slopes were significant in themselves and the difference between the slopes was calculated, and there was a significant difference between these.

There is therefore an expected difference between males and

females (as reflected in known normal values), and this was the case in every respect of the exercise, except for the values immediately after exercise. The males increased significantly more rapidly after exercise than the females, over the 100 minute period which followed.

Urea And Blood Urea Nitrogen

Figure 56 shows the values of urea and blood urea nitrogen in eight eland before exercise and immediately afterwards which were subjected to the t-test for matched observations giving t_7 = 1,503 which was not significant. The values rose after exercise over a period of 100 minutes and the difference between the value before exercise compared to the average of *all* values after exercise was significant - t_7 = 1,959*. Although the difference between the values immediately after was not statistically significant, i.e. there was no significant rise in urea values, there was a significant rise in urea over 100 minutes after exercise compared to before the chase, indicating that the rise in values is a gradual one and not necessarily apparent immediately after the chase. The increase in results was only measured over a period of 100 minutes. Urea does eventually return to normal as is seen from values taken on subsequent days. But the exact rate of return to normal has not yet been determined.

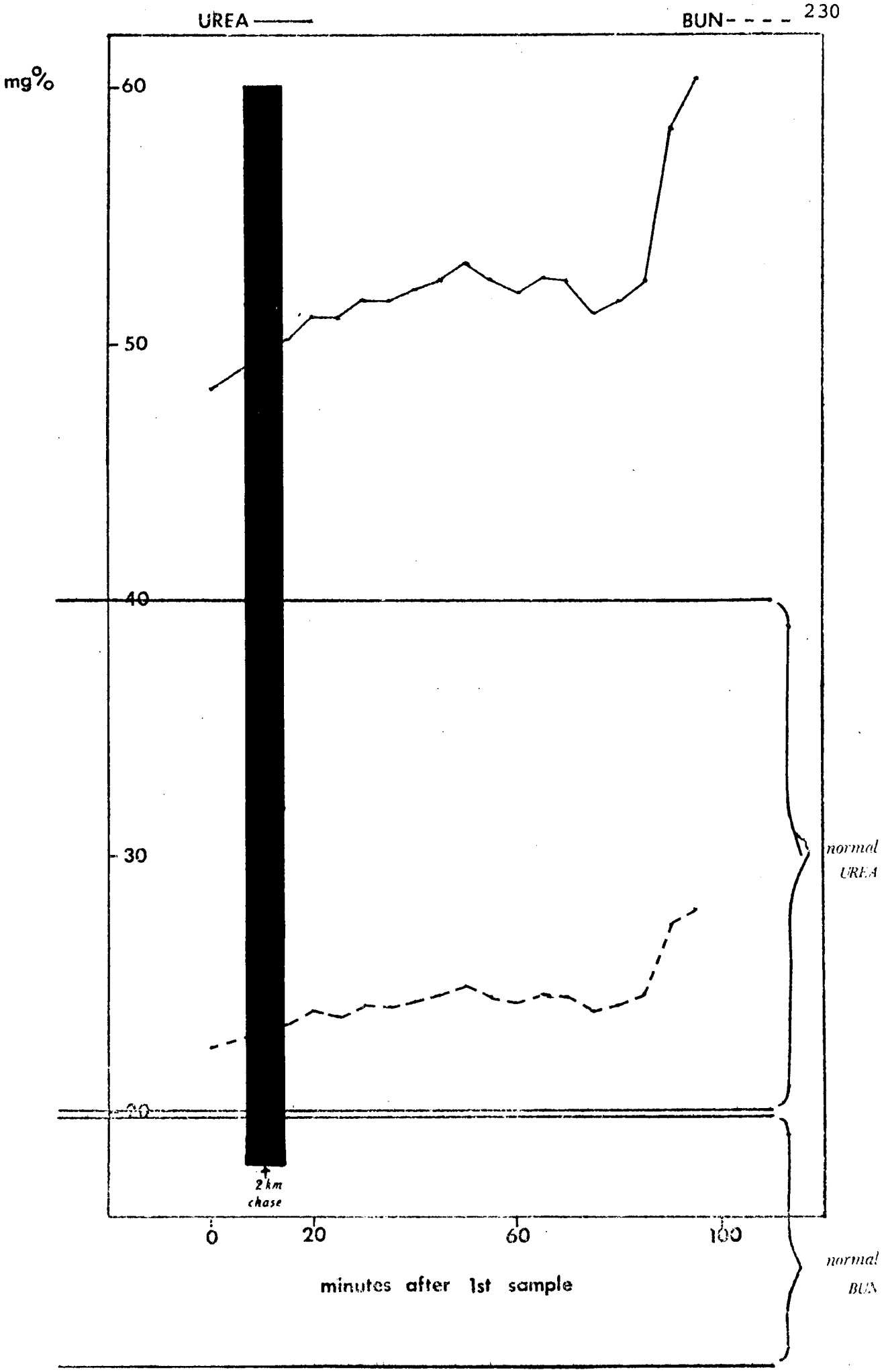


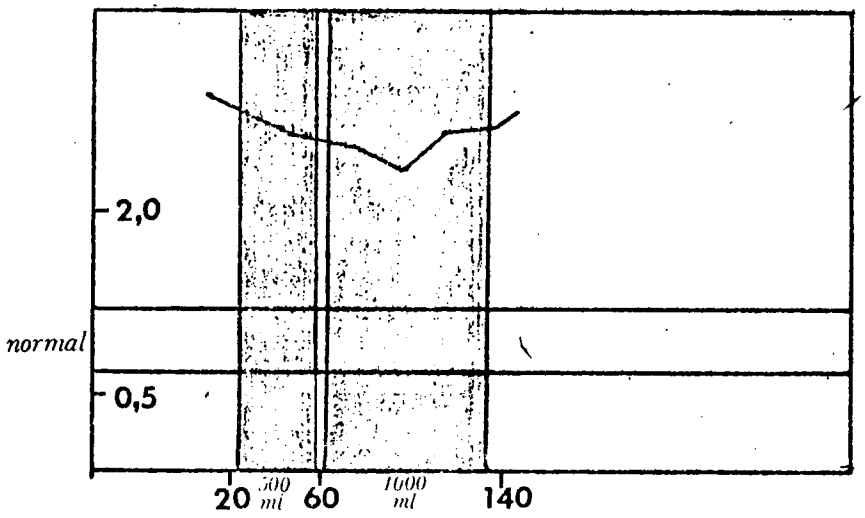
Figure 56: Urea and blood urea nitrogen levels

SABLE

CREATININE

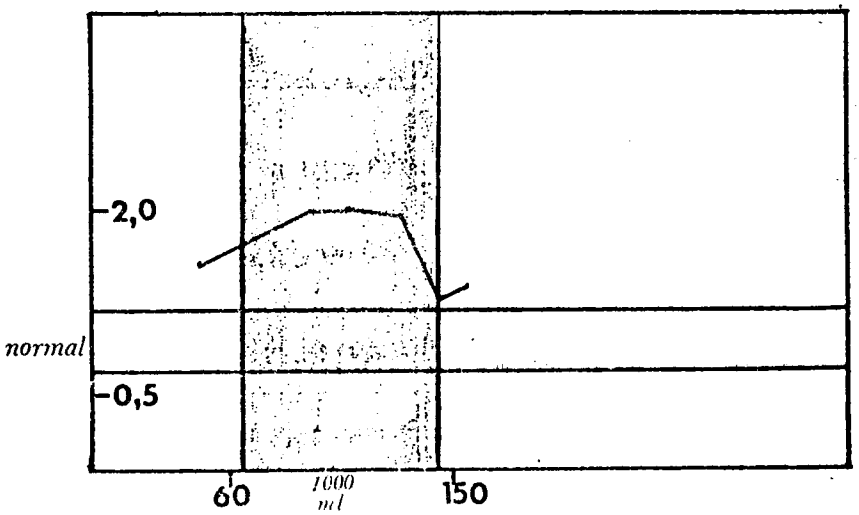
CREATININE appears to be raised by adrenaline infusion, although a time lapse occurs which permits a slow fall from the pre-infusion excitement level. The fall is followed by a rise which occurs approximately half-way through the infusion, as shown in Fig. 57A. Values also fall as a result of phenoxybenzamine hydrochloride infusion given as described in Chapter Two. Figure 57B shows a typical curve where the rise in creatinine levels is checked after about 20 minutes. After a further period of about 30 minutes, there is a steep fall in the creatinine level, probably indicating a return of kidney perfusion and a release of renal vasoconstriction. The exact time, in relation to the commencement of infusion, that this release occurs will depend, in this type of experiment, is on the blood level of phenoxybenzamine hydrochloride that is reached. There should also be a marked time-lag between a re-establishment of kidney flow and the fall in creatinine level. A more exact indication could be obtained if the phenoxybenzamine hydrochloride were to be injected intravenously separately, or even a full dose injected intramuscularly, instead of being included in the adrenaline infusion.

Figure 57C shows the results of another experiment including a sable antelope similarly treated. There was some residual excitement in spite of immobilisation so that the monitoring, and therefore the infusion, was delayed. Creatinine levels



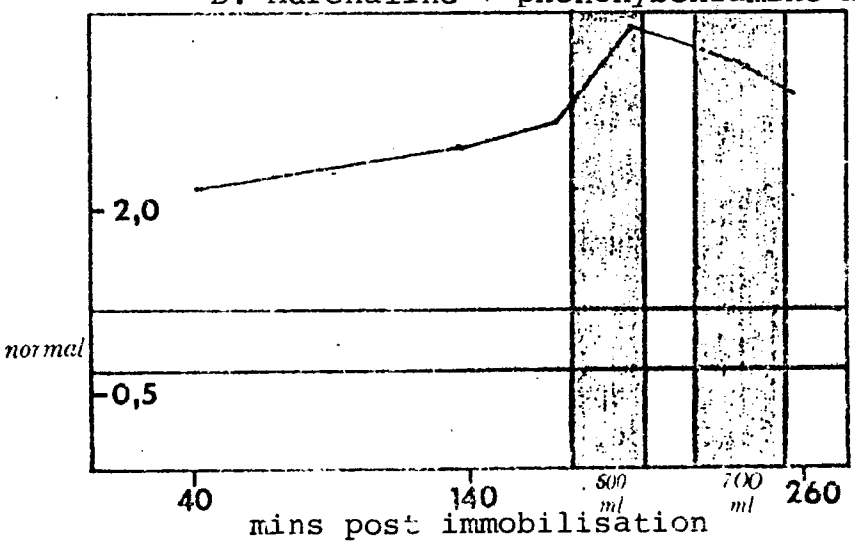
mins post immobilisation

A. Adrenaline infusion.



mins post immobilisation

B. Adrenaline + phenoxybenzamine hydrochloride.



mins post immobilisation

C. Adrenaline + phenoxybenzamine hydrochloride.

Figure 57: Creatinine levels in sable antelope after immobilisation.

showed a steady rise due to muscle tension and to endogenous adrenaline secretion, but soon commenced to fall when phenoxybenzamine hydrochloride was infused in spite of the additional adrenaline administered in the infusate.

Because the sample size was small, the Wilcoxon test of signed rank for matched observations was applied. Comparison was made between before infusion values of all sable experiments and first values immediately after start of infusion, and the latter compared to the last value before the end of infusion, in both treated (i.e. phenoxybenzamine hydrochloride added to adrenaline) and untreated groups. The results were as follows:

<u>treated group</u> (n = 3):

before and first value after: T(P,N) = 6, T(N,N) = 0

first after and last: T(P,N) = 0, T(N,N) = 6

<u>untreated group</u> (n = 2):

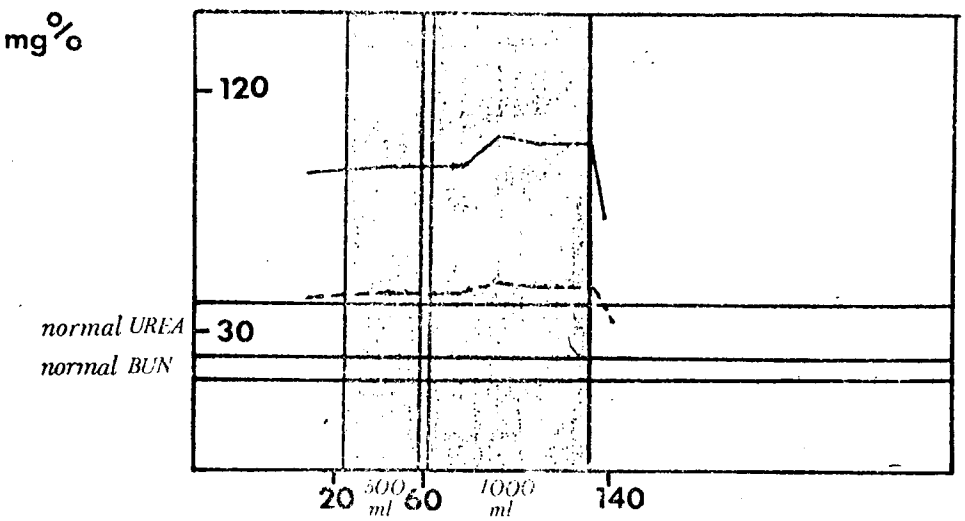
before and first value after: T(P,N) = 3, T(N,N) = 0

first after and last: T(N,N) = 3, T(N,N) = 0

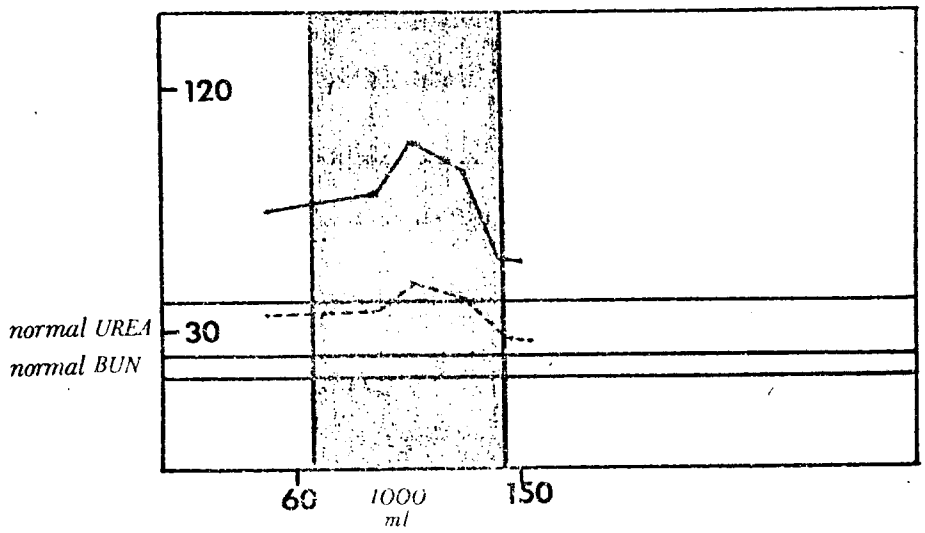
None of the results was statistically significant, the sample size was too small to compute the significance, and these results are therefore largely inconclusive.

Urea And Blood Urea Nitrogen

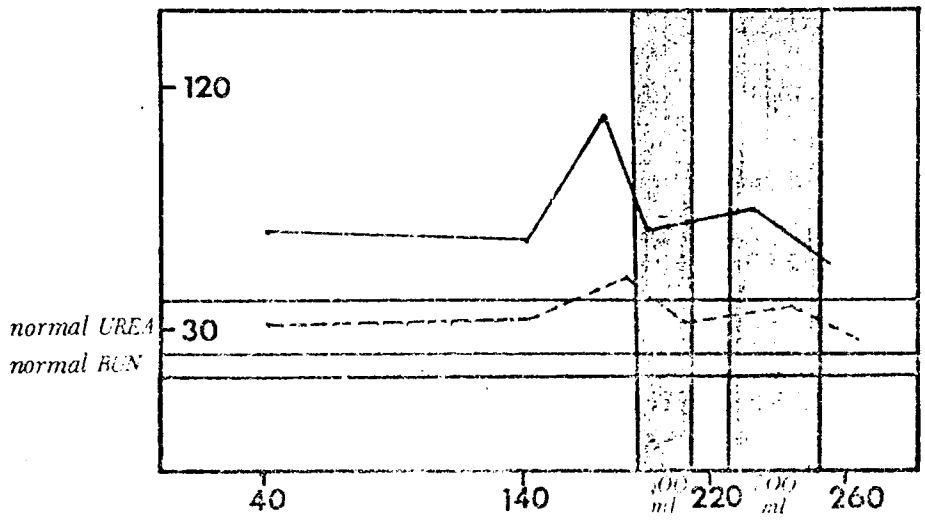
Prolonged infusion of a low level of adrenaline induces sustained medium-high blood levels of urea and blood urea nitrogen (0,33 gamma adrenaline per kg/min.). A representative curve is shown in Fig. 58 A. The initial



A. Adrenaline infusion.



B. Adrenaline + phenoxybenzamine hydrochloride.



C. Adrenaline + phenoxybenzamine hydrochloride.

Figure 58: Urea and blood urea nitrogen levels in sable antelope after immobilisation.

rise of the metabolites is associated with the sympathetic discharge while in the crush before immobilisation. When no infusion was given, both parameters fell soon after immobilisation was effected. The adrenaline infusion maintains urea and blood urea nitrogen at medium-high levels and these then drop immediately after infusion is terminated. In some instances where the infusion was prolonged, no immediate post infusion drop is seen, suggesting continuing renal vasospasm - a phenomenon found in a proportion of kidneys removed for donor purposes from live subjects or from cadavers (Chapter Fifteen). When the alpha-adrenergic blocker, phenoxybenzamine hydrochloride is added to the infusion (10 mg/litre), the levels of urea and blood urea nitrogen tend to drop when the phenoxybenzamine hydrochloride has presumably reached a certain blood level. This is illustrated in Fig. 58B, which shows a representative curve obtained from a sable antelope.

Figure 58C shows a rise in both urea and blood urea nitrogen before infusion due to adrenaline secretion during excitement. The levels are brought down as a result of infusing 500 ml saline containing 1 mg adrenaline and 5 mg phenoxybenzamine hydrochloride. During the ensuing 30 minutes, both the urea and blood urea nitrogen show a small rise which is brought down again by the infusion of a further 700 ml of infusate containing adrenaline and phenoxybenzamine hydrochloride of the same strength as before.

Both the t-test for paired values and the Wilcoxon signed

rank test for matched observations were used to analyse these results. The former test could only be applied to the untreated group - the treated group being too small. The results are as follows:

untreated group:

before and first after: $t_s = 0,0402$ $(n = 4)$
T(P,N) = 5, T(N,N) = 5

first after and last: $t_s = 0,0626$ $(n = 4)$
T(P,N) = 4, T(N,N) = 6

treated group:

before and first after: T(P,N) = 2, T(N,N) = 1 $(n = 2)$

first after and last: T(P,N) = 3, T(N,N) = 0 $(n = 2)$

None of these results was significant and the sample size was too small to apply any meaningful statistical treatment. On the whole, this set of results is inconclusive in itself. Direct observations may be made from the graphs and data taking into account the individual cases. It would appear that phenoxybenzamine hydrochloride did ameliorate the high urea levels and blood urea nitrogen, as compared to the untreated group, which showed a continued rise in urea levels after infusion with the adrenaline alone.

DISCUSSION

THE figures in this section representing the work on sable antelope infused with adrenaline solution are representative figures and do not show averages and ranges. Only nine sable

experiments have been made (one being a control experiment without infusion), the levels differing as for instance in Fig. 57B and 57C. The times of infusion in relation to immobilisation also differ, making valid comparisons difficult.

The results indicate that phenoxybenzamine hydrochloride may be effective in preventing the nephrosis syndrome. Increased renal perfusion and resulting diuresis should help to prevent blockage of renal tubules, especially if given together with alkalising substances. Stabilisation of the blood pH should also be promoted by the improved kidney function together with elimination of metabolites. The phenoxybenzamine hydrochloride should also promote peripheral blood flow and alleviate the intense vasoconstriction leading to capillary stasis and damage as described in Chapter Four. It should furthermore assist in returning to normal the pulmonary artery blood pressure which, as has been pointed out returns to only partial normal levels as a result of alleviating acidaemia (Chapter Four).

An insight into the problem of renal failure and the underlying cause of tubular necrosis has been gained in clinical medicine from the use of kidneys from cadaver donors. With the use of transplants, it became evident that the immediate function of cadaver renal allografts after transplantation was inferior to that of kidneys obtained from live donors (Pryor, Keaveny, Reed and Belzer 1971).

The period that elapsed between the death of the donor and the removal and subsequent cooling of the kidney was thought to be the critical factor, i.e. the period of warm acidaemia, but it was shown by experiments on pigs that there is little difference in function between kidneys removed at periods of five to 25 minutes after cardiac arrest (Belzer, Reed, Pryor, Kountz and Dunphy 1970).

Arteriograms of the excised kidneys showed varying degrees of vasospasm involving on occasion the entire extrarenal as well as the intrarenal arterial tree. This vasospasm could not be abolished by increasing the pressure of the irrigating fluid.

It appeared that the constriction of the kidney vessels occurred during the agonal phase preceeding the death of experimental pigs (Belzer *et al* 1970). Constriction of the renal vessels and reduction of the renal blood flow appears to be at least largely mediated by the sympathetic nervous system and may be induced through experimental haemorrhagic shock (Eckenhoff and Cooperman 1965).

Kidneys could undergo renal arterial spasm at any stage prior to final cardiac arrest. When such kidneys were transplanted they underwent severe cellular destruction which was related to ischaemic injury prior to removal from the donor, and the success of the graft could be prognosticated from the rate at which fluid could be perfused through the renal vessels after excision (Pryor *et al* 1971).

The kidney is well supplied with alpha receptors, and a sharp sustained drop in renal flow was observed in experimental dogs after haemorrhage with a fall in systemic pressure (Bell and Lister 1970).

Restoration of the blood pressure with noradrenaline infusion results in a further drop in renal blood flow. Thus the increased renal resistance offsets the increased perfusion pressure. Conversely, a drop in blood pressure induced by alpha-receptor blockade induced an improvement in the renal blood flow. It appears that the auto-regulating mechanisms of the kidney function to maintain a steady flow rate through the kidney during fluctuations of the blood pressure down to a level of 100 mm Hg. This mechanism is often absent, however, because of sympathetic vasoconstriction (Bell and Harper 1968).

The experimental animals showed a rise in blood urea nitrogen and serum creatinine as a result of prolonged adrenaline infusion at rates insufficient to affect markedly either the systemic or the pulmonary artery blood pressure. The rise in blood urea and creatinine indicates that renal function was already considerably impaired. The haemodynamic response of the kidney to small reductions of cardiac output is by efferent arteriolar vasoconstriction which thus induces normal levels of glomerular filtration in spite of reduced renal plasma flow (Merrill 1949).

When the cardiac output is further reduced and after opera-

tions involving circulatory deficiency, renal function abnormalities are likely to occur inducing a significant mortality (Grismer, Levy, Lillehei, Indeglia and Lillehei 1964, Doberneck, Reiser and Lillehei 1962).

The mechanism of renal artery constriction is complicated, involving renin as well as either neural or humoral sympathetic factors. It has been shown, however, that alpha-blockade in those patients that survived open heart surgery induced an increase in the effective renal plasma flow (Indeglia, Levy, Lillehei, Todd and Lillehei 1966). These patients already suffered from impaired renal function and high levels of circulating catecholes developed during extra-corporeal circulation especially in those that eventually failed to survive the operation.

It appears that where renal tubular blockade is likely to be aggravated by either adrenergic-induced vasospasm or by low systemic blood pressure, alpha-blockade should exert a saving effect.

CHAPTER TWELVE

LACTATE

INTRODUCTION

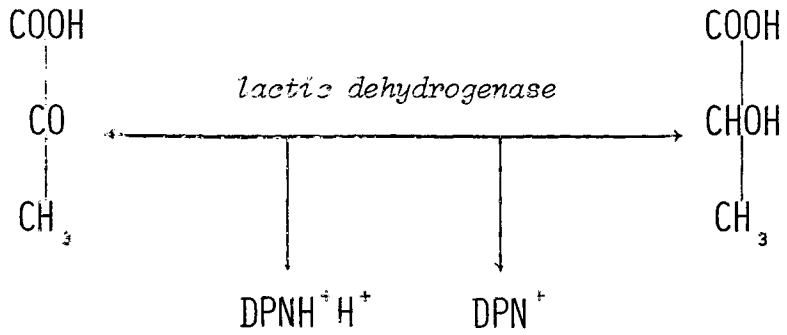
THE formation of lactic acid during intensive exercise is very largely responsible for the low pH levels found in the blood of wild animals after chasing, and there is a correlation between these two parameters (see results below). It has been shown that a respiratory acidosis hardly exists by the time the animals can be sampled after capture.

The lactic acid level in the blood during mild exercise does not rise above the resting value of 15 mg percent. The reason for this is that under these circumstances, lactic acid is disposed of as soon as it is formed, and therefore does not accumulate in muscle tissue. In more vigorous exercise the formation of lactic acid exceeds the ability of the mechanisms to oxidise or reconvert it to glycogen. In severe exercise in man, blood lactate exceeds 50 mg percent. The extent of the appearance of lactic acid in the blood appears to be related to the state of training (see also Chapter Fifteen) (Bell, Davidson and Scarborough 1961).

The fate of pyruvic acid which has been formed in this way depends upon the amount of oxygen there is available. If

sufficient oxygen is present the pyruvate is oxidised to carbon dioxide and water in the Krebs citric acid cycle. In the cell, DPN, which was reduced by taking up a pair of hydrogen atoms when the phosphoglyceraldehyde was oxidised to phosphoglyceric acid, is oxidised again by the flavoprotein and cytochrome systems and finally by molecular oxygen (Bell *et al* 1961).

If there is an insufficient supply of oxygen, oxidation of reduced DPN in this way is not possible, nor is it possible for pyruvic acid to be oxidised in the citric acid cycle. In these circumstances pyruvic acid is reduced to lactic acid at the expense of the reduced DPN.



DPN = diphosphopyridine nucleotide

This reaction, which is catalysed by the enzyme lactic dehydrogenase, is reversible and is in fact reversed when lactic acid is ultimately oxidised in muscle during recovery after exercise.

The oxidation of pyruvate by the citric acid cycle is an elaborate process which requires among other things, the presence of thiaminepyrophosphate which is derived from thiamine. In the disorder of thiamine deficiency, even quite mild forms of exercise lead to an increase in blood pyruvate because there is not sufficient co-carboxylase to bring about its rapid oxidation (Chapter Fifteen).

Skeletal muscle contracts for only a limited time without glycogen. When skeletal muscle is poisoned with iodo-acetic acid it is unable to form lactic acid but can still contract until all the phosphocreatine it contains has been broken down. Cardiac muscle, on the other hand, poisoned with iodoacetic acid continues to contract for an indefinite period, as long as it is supplied with oxygen and lactate. The energy for contraction is obtained by the breakdown of phosphocreatine, but in this case it is resynthesised. The energy for this reaction is provided by the oxidation of lactic acid. If the oxygen supply is reduced, the lactic acid can no longer be oxidised, and the heart fails when the phosphocreatine supplies are exhausted (Bell *et al* 1961).

MATERIALS AND METHODS

EIGHT eland, six tsessebe and five sable were used in this series of experiments. The procedures are described together with laboratory analyses of lactate in Chapter Two.

RESULTS

ELAND

THE level of lactic acid in test animals is well above levels reported for human athletes. Levels of 140 mg percent were established in the blood of eland run on the Percy Fyfe Nature Reserve track at an average speed of 17,85 km/h (range 12 to 22,3 - the low value being incurred by an old bull with a cardiac defect), for a distance of 2 km. Lactic acid levels for the Kruger Park animals were not established. The lactate levels of eland run on the track for 2 km at 18 km/h are shown in Fig. 59. There is considerable spread in the levels, in that certain animals were more easily persuaded to run than others, and there was some discrepancy in the size of the animals. In spite of peaks of 140 mg percent in some animals and secondary peaks of the same height, all animals showed values of approximately 60 mg percent lactic acid at 80 minutes after exercise. There was little evidence of the level of lactic acid falling below this while the animals were restrained. Levels must have fallen to normal in treated as well as control animals, as all survived and were normal at sampling on subsequent occasions. In other animals they were shown to return to normal levels within 24 hours (Fig. 60). Normal levels are 10 to 15 percent, although several animals showed elevations of blood levels at the start, which is ascribed to the restraint of sampling. The eland exhibited very little resistance to sampling, which is doubtlessly the reason that most of the

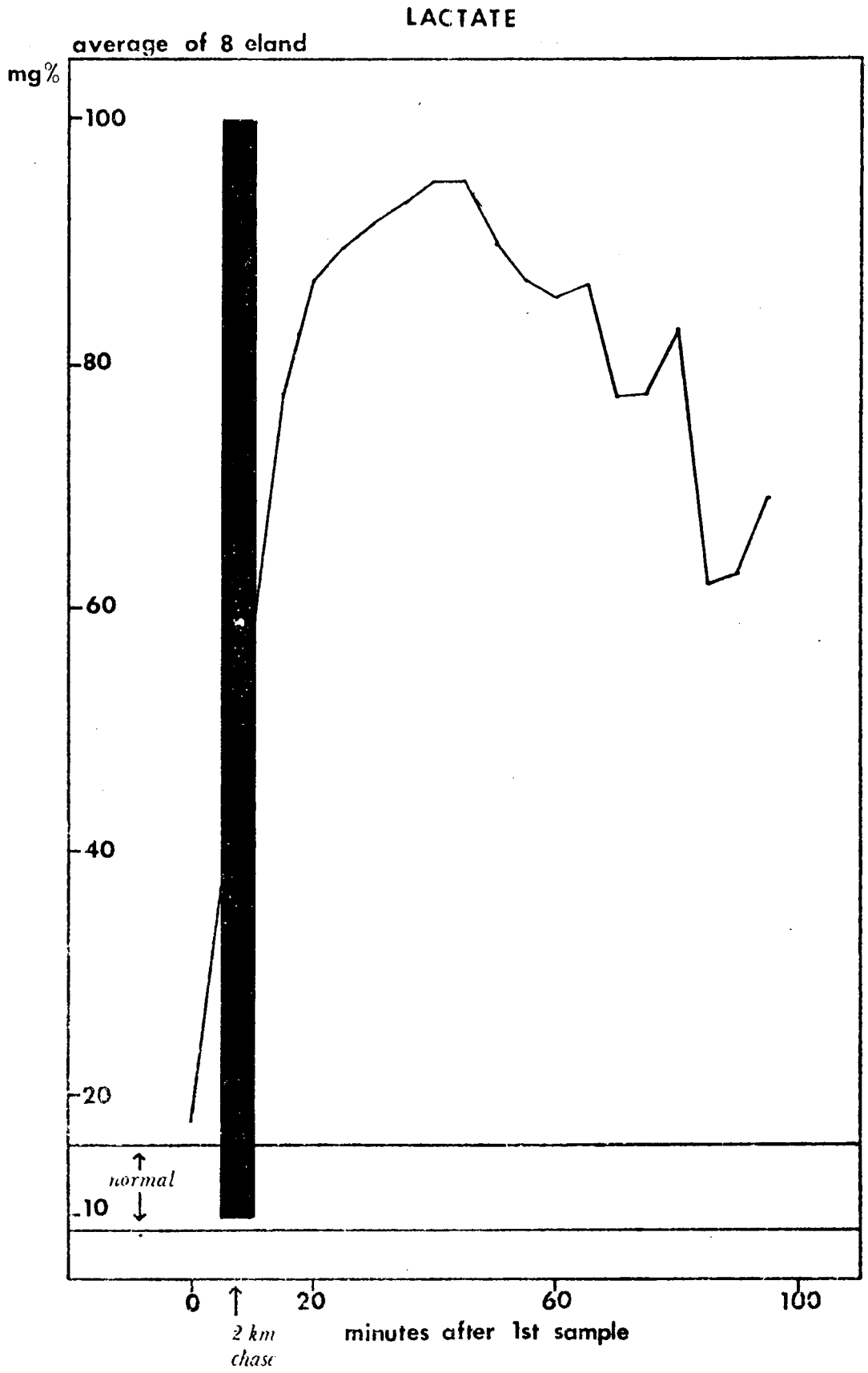


Figure 59: Lactate levels in eight eland before and after exercise.

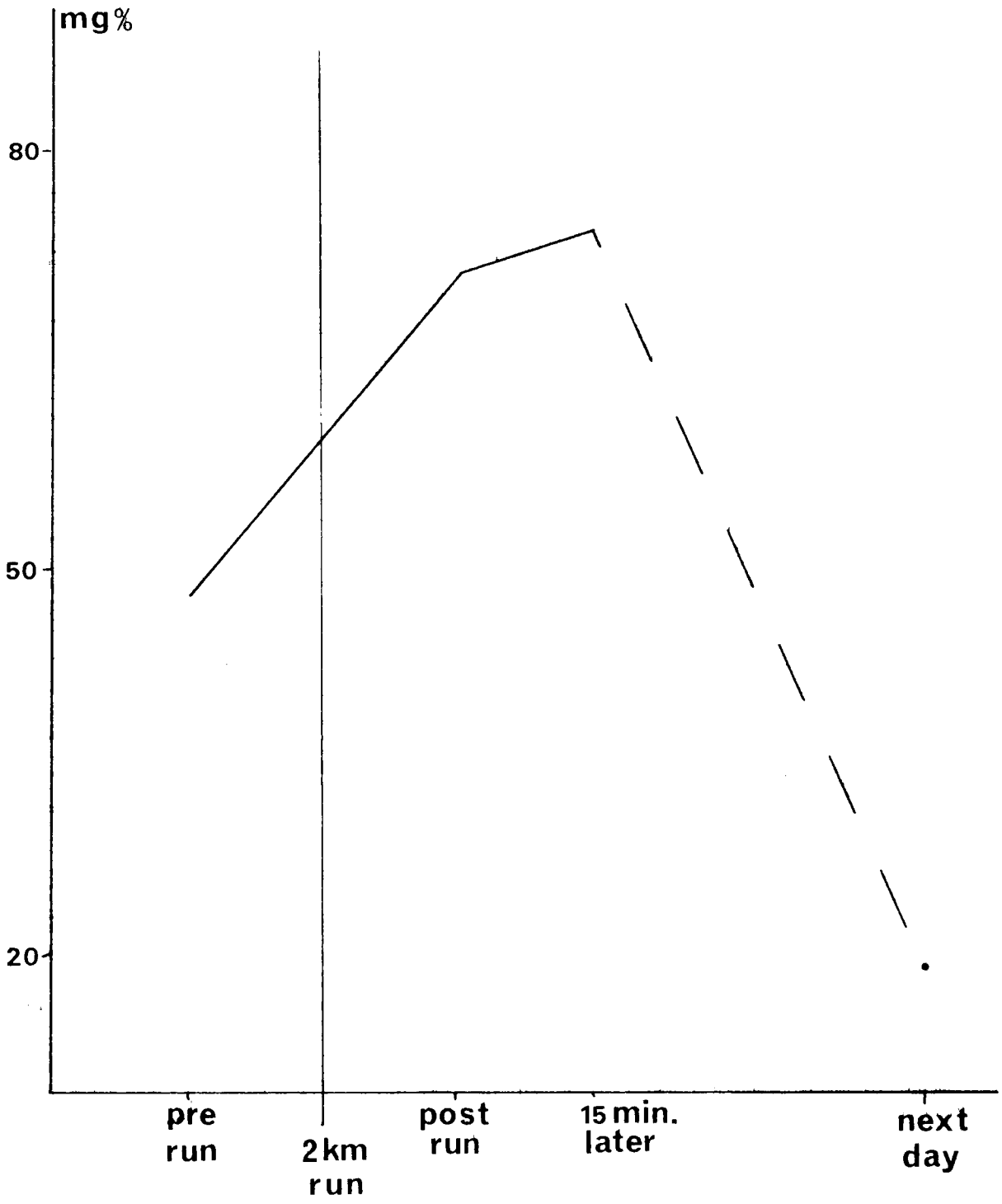


Figure 60: Lactate levels in tsessebe before and after exercise and one day later.

animals show blood levels within normal limits. This is in striking contrast to some other antelopes such as sable, where normal values are almost impossible to obtain in the unimmobilised animals. Tsessebe also show little resistance and these animals may be blood-sampled standing in a crush after they have been blindfolded, upon which they appear to become completely submissive.

Three sets of comparisons were made, as the times could not be standardised for all animals with regard to values after exercise. Therefore, the difference between values immediately before the chase and the peak values after chase were first computed using the t-test for matched observations giving a value of $t_7 = 7{,}717^{***}$. The difference between values immediately before and immediately after the chase were then compared giving $t_7 = 6{,}670^{***}$, and the difference between the values immediately before the chase and the average of all values after were computed giving $t_7 = 7{,}181^{***}$.

TSESSEBE

FIGURES for tsessebe are similar to those for eland (Fig. 61) with a greater range. The mean levels are lower, probably because the running speed of some 20 km/h is relatively slow for tsessebe (actual speed: average 23,12 km/h; range = 17,27 to 25,70 km/h). Here again, there was little tendency for the lactic acid levels to fall during restraint. The reasons for this may be many, including low liver per-

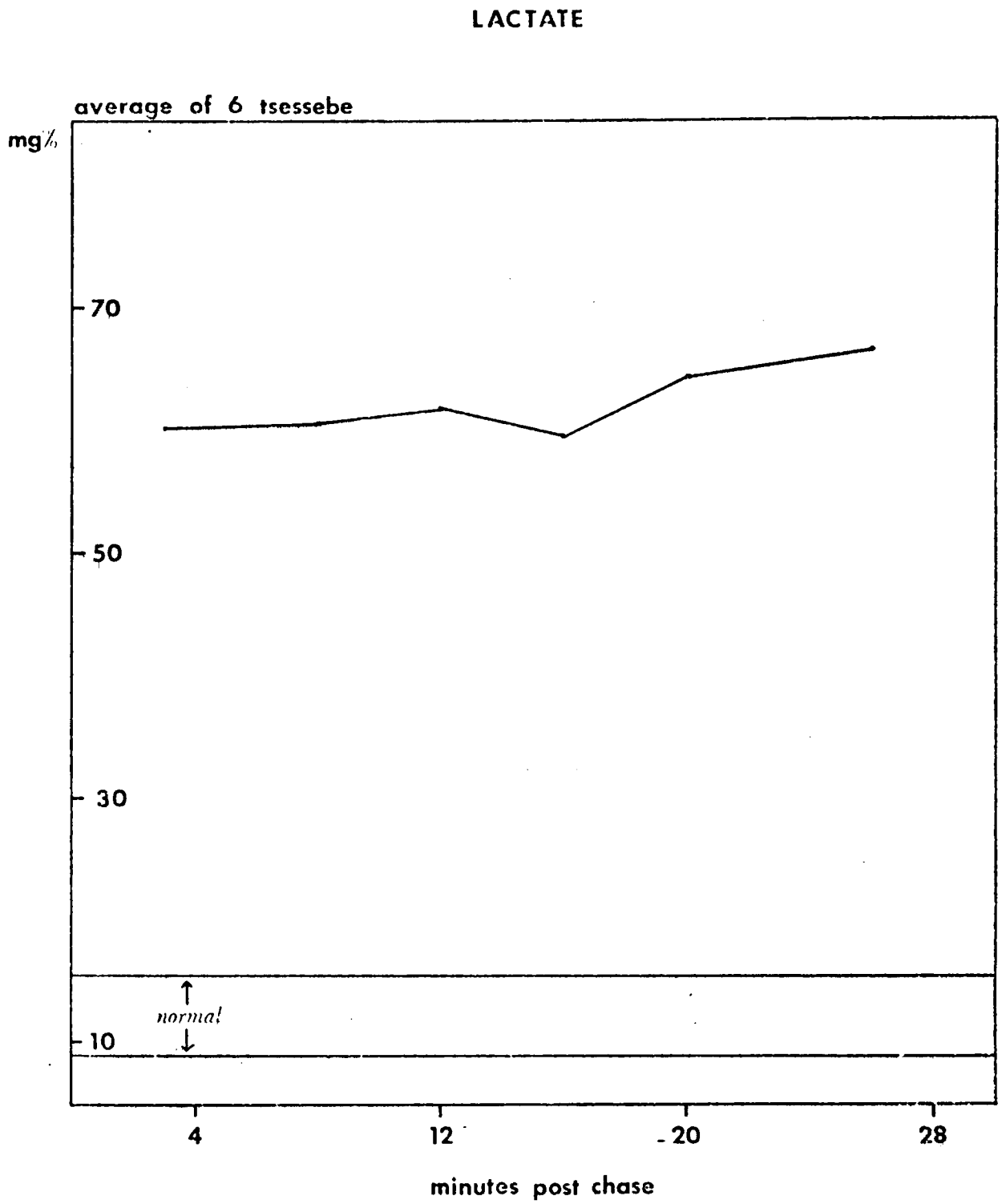


Figure 61: Lactate levels in six tsessebe after exercise.

fusion pressure, fall in cardiac output, high adrenergic discharge and muscle tension or struggling.

SABLE

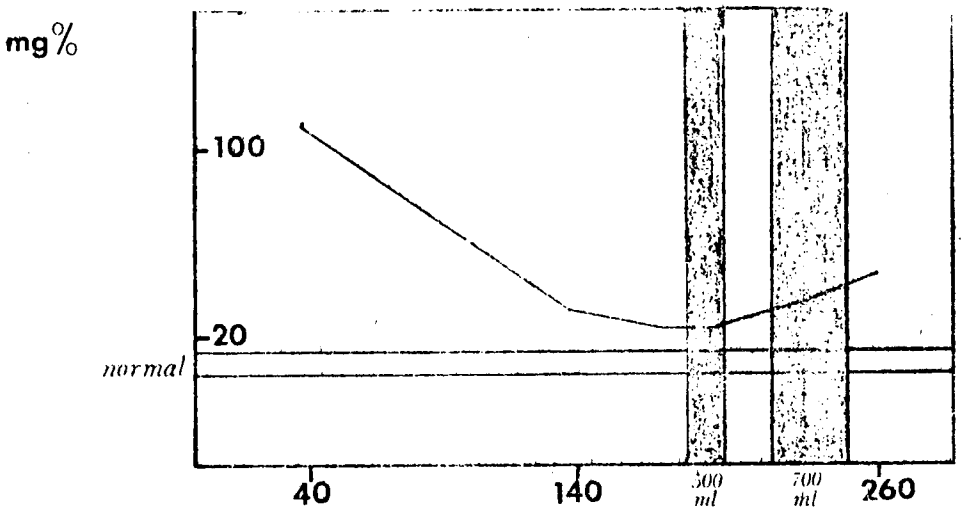
WHEN the animal is immobilised there is a slow fall in lactic levels to near normal, over a period of 180 minutes (Fig. 62A). This figure depicts a sable bull immobilised with fentanyl/xylazine/azaperone. It may be noted that there is:

(a) A steady fall of lactic acid which flattens out at 140 minutes at 30 mg percent at just above normal, and

(b) that adrenaline infusions at a rate of 20 µg per kg per hour caused the lactic acid levels to be maintained. Potassium levels also rose (Chapter Thirteen).

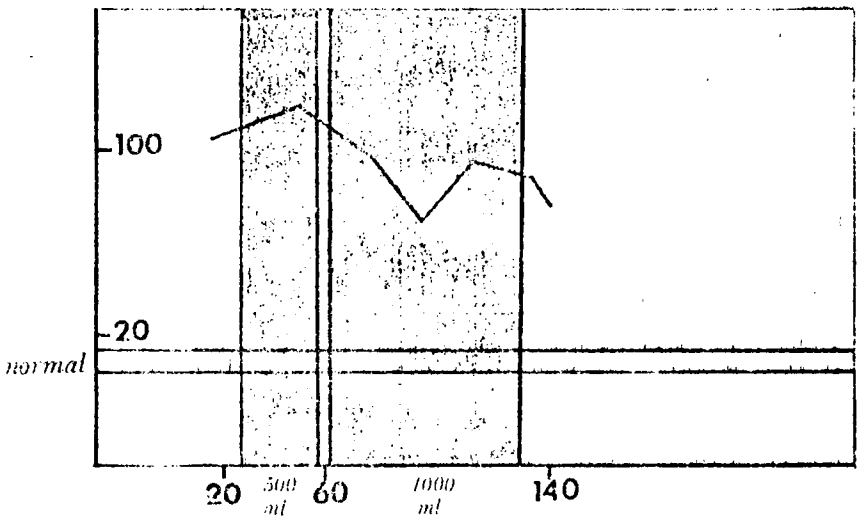
The original high level of lactic acid was no doubt due to struggling in the crush during injection, the sable being inclined to fight and struggle to a far greater extent than the eland and the tsessebe.

Adrenaline infusion in the immobilised animal does not, in itself, raise the lactate to high levels. Figure 62B shows how the lactic acid falls in spite of infusion from the level of 100 mg percent, to a varying level of 80 mg percent. It thus appears that the adrenaline will raise the lactic acid level only to a certain medium height. The results shown in Fig. 62B suggest that continuous adrenaline



mins post immobilisation

A. Adrenaline + phenoxybenzamine hydrochloride.



mins post immobilisation

B. Adrenaline infusion.

Figure 62: Lactate levels in sable after immobilisation.

infusion will tend to maintain lactic acid levels at medium levels for a longer period than in non-infused animals (see Fig. 62A) but that eventually the lactic acid level falls if the infusion is maintained for a longer period, e.g. 120 minutes at a steady flow rate. For further explanation of the rationale of the tests on sable, see Chapters Eleven and Thirteen.

It may be noted that the addition of a small amount of phenoxybenzamine hydrochloride (alpha-adrenergic blocking agent) to the adrenaline infusion did not appear to influence the formation of lactic acid (Fig. 62A). A similar pattern for adrenaline with phenoxybenzamine hydrochloride infusion is shown in Chapter Fifteen.

Comparing values before and immediately after the start of the infusion statistically, in both treated and untreated groups gave the following results:

Untreated group:

before and first after: $t_4 = -0,430^{**}$ ($n = 5$)

first after and last: $t_4 = -2,214^{*}$ ($n = 5$)

Treated group:

before and first after: $t_1 = -9,947$ not significant ($n = 2$)

first after and last: $t_1 = 99,0^{***}$ (but see Wilcoxon) ($n = 2$)

The treated group was considered too small for the t-test to have any meaningful application and so Wilcoxon's signed rank test for matched observations was used, and the

following result obtained.

before and first after: $T(P,N) = 2$, $T(N,N) = 1$ not significant

first after and last: $T(P,N) = 0$, $T(N,N) = 3$ not significant

Actually, even the Wilcoxon tests could not be probed for significance, as the results fell outide the range tabulated for significance. As far as statistical significance is concerned, these results are inconclusive due to the small sample size.

Lactate Compared To pH

The correlation of lactic acid and pH for eland is shown in Fig. 63. The drawing shows pH plotted against lactate. The curve is fairly constant and shows a steady fall in pH as the lactate levels rise. Minor variations in the curve can be ascribed to changes in carbonic acid/bicarbonate values in relation to periods of agitation and respiratory response. The correlation coefficient was calculated between lactate and pH as follows:

$r = -0,659^{*}$ (closer to **) one-sided ($n = 7$) and the regression line is formulated as follows:

$y' = -0,00324x + 7,3051$.

There is a significantly negative correlation between pH and lactate, i.e. as lactate levels rise the pH falls.

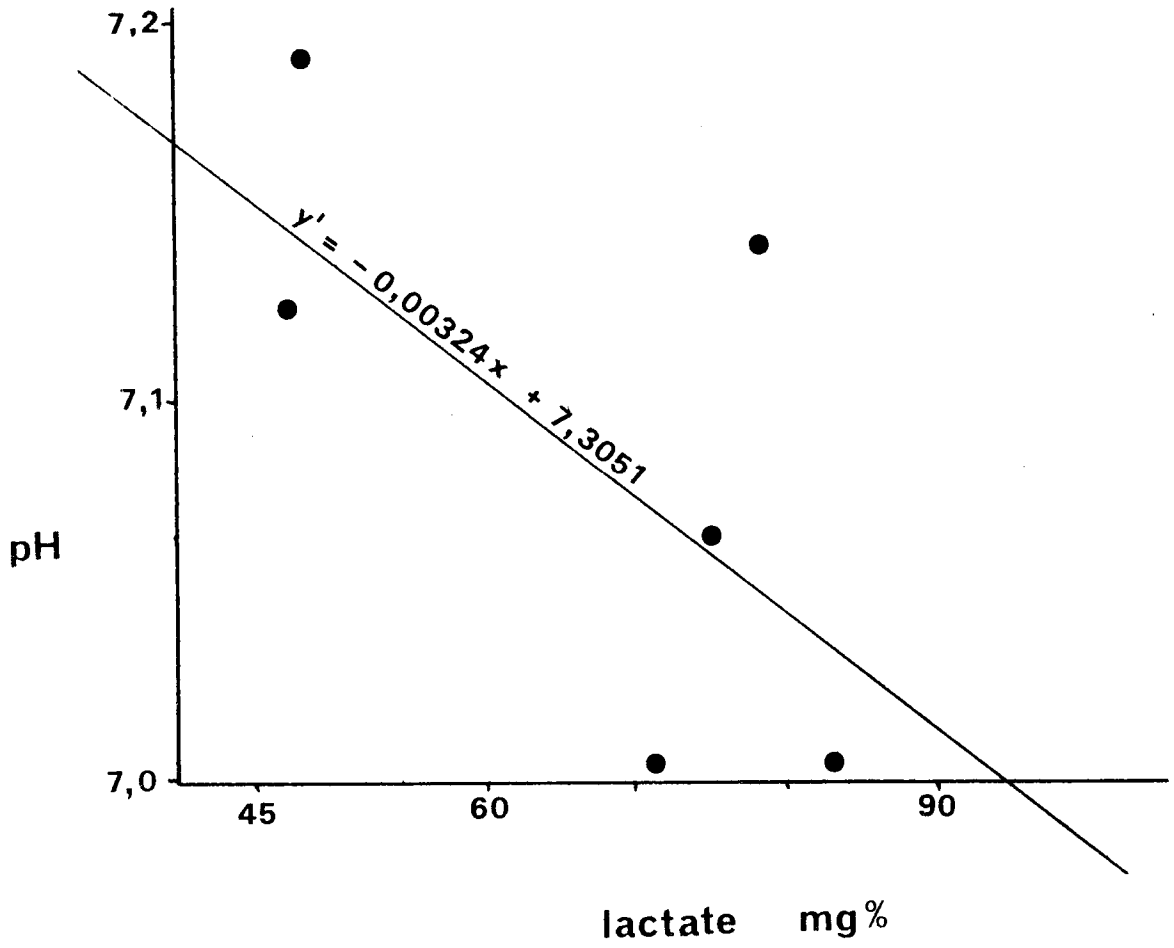


Figure 63: Lactate compared to pH values showing regression line of values for eland after exercise.

DISCUSSION

THE survival of animals show that the levels of lactic acid shown by eland and tsessebe after the 2 km chase at medium speed, does not cause the necrosis of tissues and coagulation of muscle protein which is associated with excessive exercise, and presumably, at least partly, from excess lactic acid formation. These effects were seen on our zebra at autopsy. These animals had, as explained, run faster and exhibited a lower blood pH than the eland and tsessebe run on the Percy Fyfe track.

The lactate levels were also lower in these animals than in springbok captured in drop nets (Gericke and Hofmeyr 1976). It is unlikely, however, that specific levels of lactate will be found to be an indication of lethal levels of capture stress, but rather the ability of the animal to reduce these levels. Thus a further drop in pH was observed during the first hour after capture in both zebra and wildebeest, also it was found that the blood pH did not accurately reflect muscle acidity. There may also be considerable species differences in the ability of the heart to continue to function under the effects of low pH, and other associated factors such as high potassium levels and adrenaline effects. It seems probable that the track-exercised animals not only exhibited lower lactate levels (it is established that pH levels were higher) than the free-living animals, but also exhibited lower sympathetic tonus during handling and the immediate post capture period;

as far as could be deduced from struggling, and from flight reaction, i.e. a certain amount of training and familiarisation with the procedure had already taken place (see also Chapter Fifteen under 'Preventative Measures').

CHAPTER THIRTEEN

POTASSIUM AND CALCIUM

POTASSIUM

INTRODUCTION

IN protein breakdown during stress or inflammation, three m-equiv. potassium are released from red cells and muscle fibres, for each gram of nitrogen. The potassium in red blood cells is 90 m-equiv./l so that haemolysis of 1 litre of blood of 50 percent haematocrit released 45 m-equiv. potassium according to Davidsohn and Henry (1969). These authors point out that in visible haemolysis in plasma, the haemoglobin concentration ranges between 100 to 200 mg/100 ml. This is about 5 m-equiv. potassium per litre for the higher level of haemoglobin thus bringing the level of the potassium to about 9,5. However, much higher levels of plasma haemoglobin may occur, or similar levels of myoglobin representing release of potassium from muscle cells.

Cardiotoxicity tends to occur at levels exceeding 6,5 m-equiv./l, and a level of 7,5 m-equiv./l has definite toxic effects on the heart. Haemolysis and damage to muscle fibres are not the only source of raised blood potassium. A rise in pH of the blood and extravascular fluid will induce a movement of potassium from the cells into the

bloodstream. Plasma potassium rises about 0,6 m-equiv./l for each 0,1 unit fall in blood pH. If the blood pH is 7 and the plasma potassium is 7 m-equiv./l, the plasma potassium will fall to a normal level of 4,6 m-equiv./l when the blood pH is restored to normal (Davidsohn and Henry 1969). It has been pointed out (Gericke and Hofmeyr 1976) that treatment of acidosis by intraperitoneal sodium bicarbonate solution (1 litre of a 1,4 percent solution) in sheep overcomes metabolic acidosis allowing potassium to re-enter the cell.

Most of the electrocardiogram tracings taken after forced exercise or struggling, show evidence of a hyperkalaemia typified by an excessively high T-wave. The harmful effects of high potassium on heart function are well known (Guyton 1966). Excess potassium in the extracellular fluids causes the heart to become dilated and flaccid and also - other things being equal - shows the heart rate. At such levels potassium can also cause atrioventricular block. If the potassium is elevated to 8 to 15 m-equiv./l, which is only two to three times the normal value, it will weaken the heart to such an extent that it will cause death. The effects of potassium excess are believed to be caused by a decreased resting membrane potential arising from high potassium concentration in the extracellular fluids. As the membrane potential decreases, there is a similar decrease in the muscle potential thus making the action of the heart progressively weaker. A deficiency of calcium ions also causes cardiac flaccidity similar to the effect of potassium. As pointed

out above, it is believed that the calcium ions in the blood are depressed during high lactate values, and therefore low pH values.

Materials And Methods

Potassium levels have been measured in two sable antelope so far. This experiment was conducted concurrently with blood metabolite and lactate determinations for which nine sable were used. The procedure and laboratory analysis are described in Chapter Two.

Results

A correlation coefficient and regression line were calculated for one set of values. Over a period of 267 minutes, the potassium levels rose from 53 mg percent to 107 mg percent in six observations. The regression line equation is as follows:

$y' = 0,253x + 34,43$ and the correlation coefficient is

$r = 0,935^{***}$

It must be borne in mind that although the correlation coefficient (rise of potassium with time) is statistically highly significant, the potassium levels have only been analysed in one sable antelope after immobilisation. The rise of potassium is consistent as can be seen from the regression line, and rises well above normal values (normal values in human serum = 16 to 22 mg percent) (Fig. 64).

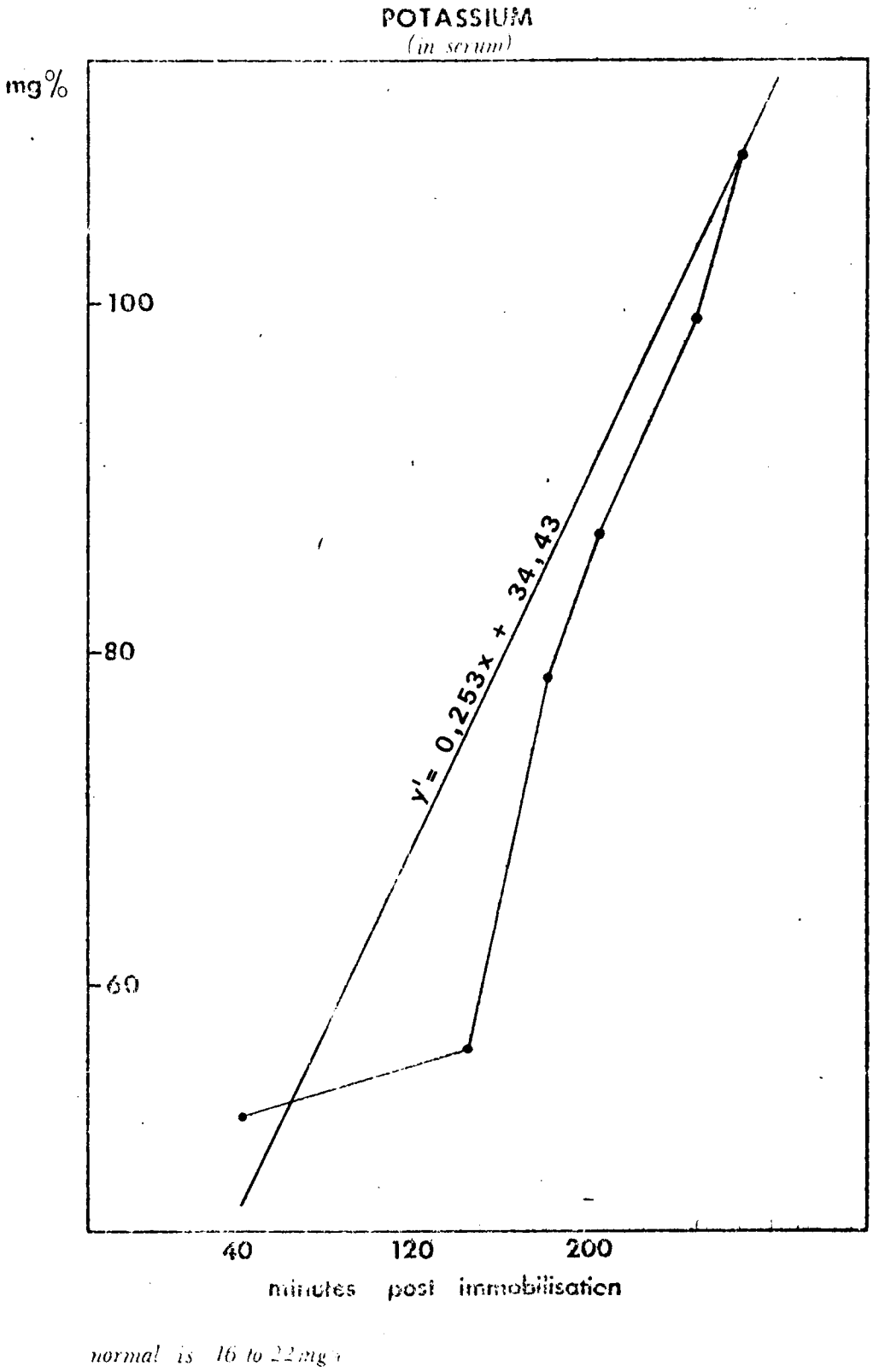


Figure 64: Potassium levels in sable antelope after immobilisation.

DISCUSSION

THE presence of hyperkalaemia was originally postulated from the presence of myoglobin and haemoglobin in the plasma, and confirmed from the electrocardiogram tracing. Under this condition there is a marked advantage of sodium therapy as this tends to redress this condition. As a result of infusing 1 000 m-equiv. sodium bicarbonate to a 250 kg animal, the sodium level is raised by about 50 m-equiv./l. The urinary excretion of sodium and potassium is in the proportion of 2 parts sodium to 1 part potassium; eventually high sodium is excreted selectively through the aldosterone mechanism.

Furthermore, there is an immediate dilution factor. If one litre is administered fairly rapidly to a 250 kg animal of which approximately 1/12 of 8 percent is the capacity of the circulating blood, the dilution of the blood approaches 5 percent and the plasma is 10 percent, assuming an haematocrit of approximately 50 percent, and the reduction in potassium may well represent a major part of those factors responsible for improved cardiac function resultant on bicarbonate infusion. Finally, as explained above, each 0,1 unit fall in blood pH will reduce the potassium level in the plasma by approximately 0,6 m-equiv. as the potassium moves back into the cells.

It may be noted that a lowered potassium may occur later due to continued stress, resulting in an increased aldosterone

output and sodium retention. High potassium levels (25 percent increase of one m-equiv. in plasma) will increase the aldosterone output resulting in increased potassium excretion. When aldosterone increases sodium reabsorption in the distal tubules, it at the same time increases potassium secretion, so that an exchange of action occurs, sodium ions being reabsorbed and potassium ions being secreted. Excessively low potassium ions should also be guarded against as a pathological condition associated with intestinal stasis or reduced intake in anorexia. Potassium is more important than sodium in the maintenance of acid-base balance, as potassium bicarbonate is the primary intracellular inorganic buffer.

In potassium deficiency there is a relative deficit of intracellular potassium bicarbonate so that intracellular acidity rises. The respiratory centre responds by lowering PCO_2 through hyperventilation. The renal cells respond by retaining sodium and potassium and excreting hydrogen ions so that through these two mechanisms an extracellular alkalosis is produced.

Lack of potassium causes drowsiness, muscular weakness and mental confusion. Electrocardiogram changes show depression of the ST-segment. The T-wave may become flattened or inverted. There is a fall in blood pressure and intestinal atony may occur. Normal levels of potassium are approximately 16 to 22 mg/100 ml blood serum. Levels in a sable that struggled before immobilisation were already 57 mg percent.

After four-and-a-half hours of light immobilisation with some struggling, the level had risen to some 107 mg percent (16 m-equiv.). This is well within the lethal level even without additional pejorative factors such as low pH, high catecholamines and possible low calcium ion levels.

The sable mentioned (Fig. 64) did not show the extensive discolouration of the serum denoting intravascular haemolysis. The potassium levels in those animals from previous tests that showed a high degree of discolouration with high levels of haemoglobin and myoglobin in the serum are likely to be even higher and the extent to which serum potassium levels constitute a lethal factor becomes provocative. The readiness of animals such as the sable to show red discolouration of the serum when restrained in a crush or chased and the association of this discolouration with potassium suggests that these animals are likely to succumb readily from hyperkalaemia after capture, especially if subjected to handling and being restrained by hand.

CALCIUM

INTRODUCTION

CALCIUM ions are generally accepted to be essential for muscle contractility, and laboratory experiments showing how cardiac contractions fail if the heart is perfused with calcium deficient fluid are too well known to need specific

reference. Less well known are the conditions under which calcium ions in the circulating blood become deficient.

Contraction of skeletal muscle, and cardiac failure in diastole, occurs with the rapid infusion of sodium citrate, a standard *in vitro* anti-coagulant; in calcium drain during lactation; in parathyroid malfunction; during calcium administration in pronounced rickets, and in alkalotic states.

Contractile activation of heart muscle normally involves a transient calcium current associated with the action potential (Miller 1974). In fact, under certain conditions, calcium ions can induce a contraction without association with membrane potential change after the muscle fibre has been depolarised with potassium. The mechanism of this effect has been postulated to be a calcium release from the store in the sarcoplasmic reticulum by calcium influx. Similarly a prolonged restoration period obtains following a reduction of calcium, with a decline of peak tension, indicating that calcium stores are not rendered available under conditions of reduced serum calcium.

Recent experiments have indicated more subtle influences. Chloride ions are responsible not only for carrying a chloride current, but play a role in the regulation of calcium conductance availability. This, it is suggested, is due to an influence of chloride ions on the electrical field at the internal face of the muscle membrane (Mounier and Vassort 1974)(see also Gordon (1976) for response to calcium influx).

It is not suggested that chlorine ions play a significant part in decreased muscle activity in the stressed animal. The clearly demonstrated reduction of cardiac function, however, renders the investigation of changes in circulating ions as a possible mediating factor of considerable importance.

RESULTS

A depression of calcium ions after exercise has been postulated from the electrocardiograph tracings. Typical slurring of the QRS-complex indicates a calcium ion deficiency (Volkart 1957) (see Plate 7).

DISCUSSION

THE effectiveness of calcium ions may be depressed by the presence and effect of lactic acid. We know (Chapter Four) that the rise in lactic acid and fall in pH in exercised wild animals tends to be excessive under conditions of capture. Levels of about 15 millimdles per litre which is consistent with maximum muscular exercise within normal ranges appears to be sufficient to depress the serum ionised calcium combining it into a physiologically inactive form. Thus the lactic acid has a double pejorative effect on heart muscle (Pitts 1969).

(a) The contractility of heart muscle, the positive inotropic effect and therefore by inference the cardiac output is reduced.

(b) The available calcium ions are also reduced and therefore the contractile power of the cardiac muscle fibres is still further reduced.

If the calcium content of the blood is increased sufficiently to saturate the binding capacity of the lactate, so that the calcium of the blood is restored, the cardiac action should improve. This fact has, however, been difficult to establish with any degree of certainty in the acutely stressed animals. Only qualitative indications of improved recovery were formed in those subjects infused with sodium bicarbonate and 'Normosol' solution, as compared to those given sodium bicarbonate in saline. The widely differing circumstances of each animal makes conclusions uncertain. Also the paucity of animals on each trial has militated against the use of control animals, or tests using a balanced electrolyte solution containing calcium ions without bicarbonate. In any case a specifically balanced solution with an excess of calcium is probably indicated where a possible deficiency obtains.

The pronounced amelioration of clinical symptoms that occurs upon the infusion of sodium bicarbonate dissolved in either saline or 'Normosol' makes this question of the exact mechanism operating in conditions of acute stress of secondary importance. The relationship of chronic stress and calcium deficiency in wild, and indeed also in domestic animals during transport, appears to be highly relevant and this is to be discussed under the General Discussion (Chapter Sixteen).

CHAPTER FOURTEEN

TRACE ELEMENTS

SELENIUM

INTRODUCTION

ACTIONS IN THE BODY

LACK of selenium in the diet causes various deficiency diseases such as muscular dystrophy, cardiac degeneration and exudative diathesis. Experimentally, rats on selenium-deficient diets show necrotic liver degeneration. Selenium appears to be identical to the 'Factor 3' originally prepared from kidney powder, and which is essential for preventing hepatic and muscle lesions (Schwarz, Porter and Fredga 1972). It is associated with the metabolism of vitamin E, but the lesions of selenium deficiency can generally not be prevented by vitamin E administration. On the other hand, symptoms of vitamin E deficiency such as liver necrosis in rats could be prevented by giving selenium. As under normal conditions, the tissue concentrations of selenium are lower by one order of magnitude than those of vitamin E, it can be assumed that the biologic role of selenium and its derivatives in vertebrates is catalytic (Walter, Schwartz and Roy 1972). These authors adduce evidence that the administration of selenite and selenate results in selenium-containing protein fractions

which may be isolated from experimental animals, and that these proteins contain selenium isologs of their natural sulphur amino acid constituents, the seleno-amino acid moieties forming reactive centres for these proteins. Thus the retardation of lipid oxidation by selenium-rich fractions has been equated with reductive properties of selenoproteins (Hamilton and Tappel 1963).

Low Selenium Levels And Capture Myopathy

The effects on the animal body under clinical conditions from eating so-called dystrophic hays is discussed in Chapter Sixteen. It is clear that the wild animals that we are dealing with do not suffer from frank selenium deficiency or from clinical manifestation of cardiac lesions or liver necrosis before they are disturbed, i.e. under natural conditions. There appear to be no reports and no experimental data to indicate whether sub-clinical deficiencies may exist that may predispose the animals to muscular dystrophy, cardiac lesions, or liver degeneration on being subjected to capture stresses. This is an important point which should be investigated, particularly in relation to the drop in liver selenium values which has been established as occurring at a time of the year when the herbage is commencing to grow, and possibly also in short supply. It is now being established that other essential trace elements such as cobalt and copper follow a similar pattern.

Nutritional myopathy due to selenium deficiency has been recognised as an important factor in domestic livestock

(Buchannan-Smith, Sharp and Tillman 1971). The possibility of selenium deficiency has also been investigated in animals in zoological gardens and it is believed that overt myopathies are likely to occur when animals from selenium deficient areas are subjected to stress (York 1974). Paradoxically the danger of this type of myopathy developing during transit is increased now that improved methods of chemical capture and tranquillisation have been developed; these methods facilitating the relocation of animals immediately from the place of capture to far-off destinations without prior resting and feeding on concentrated foods. Low levels of mineral elements have been correlated together with the effect of season on the breeding cycle of plains antelope in the Transvaal highveld by Skinner, van Zyl and Oates (1974). Low levels of trace elements have also been implicated as a factor in high calf mortality occurring in roan and sable antelope in the Transvaal (Wilson 1975).

Materials And Methods

Selenium levels in normal biological tissue range from 0,005 to 0,5 p.p.m. with the result that only ultramicromethods can be used for selenium analysis. Colorimetry and fluorimetry have been utilised for the quantitative analysis of selenium in biological materials (Cheng 1956, Hoffman, Westerby and Hidiroglou 1968). A more modern analytical technique, neutron activation followed by a high resolution gamma-ray spectrometry, has also been used extensively for the analysis of trace elements in biological

tissues because of its inherent high sensitivity for many elements (Turkstra, Retief and Cleaton-Jones 1975).

Five stable isotopes of selenium exist in nature. Table 19 gives the relevant nuclear data for radionuclides which are produced from selenium by neutron activation. Only three of these radionuclides, namely ^{75}Se, ^{77m}Se and ^{81}Se can be obtained with high specific activity. Neethling, Brown and de Wet (1968) employed the short-lived radionuclide ^{77m}Se for the instrumental radioactivation analysis of selenium in biological material. Because of possible interferences associated with this fast technique, satisfactory results cannot generally be obtained at very low concentrations, unless a thin NaI(Tl) scintillation crystal or a Ge(Li) detector is applied (Blotcky, Arsenault and Rack 1973). The virtually pure beta emitter ^{81}Se has been used to a limited extent (Bowen and Cawse 1963). The radionuclide most commonly used for the determination of selenium in biological material is ^{75}Se (Steinnes 1967). A limitation of the use of this radionuclide is the length of irradiation time required to achieve a high activity, but the long half-life ($t_{\frac{1}{2}}$ = 120 days) allows sufficient time for careful radiochemical separation and activity measurements. It was therefore decided to investigate the use of high-resolution gamma spectrometry for the direct determination of selenium in liver samples of wild animals at regular intervals over a period of several years, 25 months of which have now been completed.

Table 19: Nuclear data for radionuclides produced from selenium by irradiation in a thermal reactor.

target isotope	abundance (%)	activation cross-section (barn)	product isotope	half-life	gamma-ray energy (keV)	activity of Se †
^{74}Se	0,87	30	^{75}Se	120 d	97 121 136 199 265 280 305 401	25*
^{76}Se	9,02	22	^{77m}Se	17,5 s	162	97*
^{78}Se	23,52	0,38	^{79m}Se	3,9 m	96	2,9
		0,05	^{79m}Se	$6,5 \times 10^{4}$y	nil	-
^{80}Se	49,82	0,08	^{81m}Se	57 m	103	1,5
		0,53	^{81}Se	18,6 m	1% gamma 272 280 550 560 830	25*
^{82}Se	9,19	0,05	^{83m}Se	69 s	350 650 1010 2020	0,46
		0,004	^{83}Se	25 m	225 358 520 710 833 1060 1310 1880 2290	0,04

† after activation for one half-life $0 = 1 \times 10^{12} n\ cm^{-2}\ sec^{-1}$ (mC/g)

From Harthoorn and Turkstra (1976)

Preparation of the liver samples, standards, irradiation and measurement of gamma activity is discussed in Chapter Two.

Liver samples from a total of 74 animals have been analysed for selenium, and are being analysed for other trace elements.

These consist of eight species from five main areas. The most numerous among these are warthog (49). Other species include impala (7), springbok (13), white rhinoceros (1), blue wildebeest (1), buffalo (1), nyala (1) and black wildebeest (1).

RESULTS

A gamma spectrum of a liver sample after 90 seconds of irradiation and 20 seconds of decay time is shown in Fig. 65. The 162 keV photopeak of ^{77m}Se is well separated from other gamma photopeaks. No interference due to the 198 keV photopeak of ^{19}O can be observed. It was observed by radioactivation that the 'Bovine Liver Standard' used contains 1,14 µg of selenium per gram of sample compared to the prepared selenium reference standard. Figure 66 shows the seasonal variation in selenium content in the liver of all animals. Figure 67 shows the values over the same time period for males only, regardless of species or location. Both curves reflect chiefly grazers. It may be assumed that the impala (facultative browsers) were taking a quantity of browse at least during the winter. In spite of this, the impala liver values do not exceed those of the warthog which are classed as grazing animals. Figure 68 reflects the values in all animals but with lactating and pregnant females shown separately. The accuracy of the analysis has been calculated to be approximately 2 per-cent (0,97 to 1,02 in five identical samples) which is ade-quate to cover the wide range of values which exceed 200

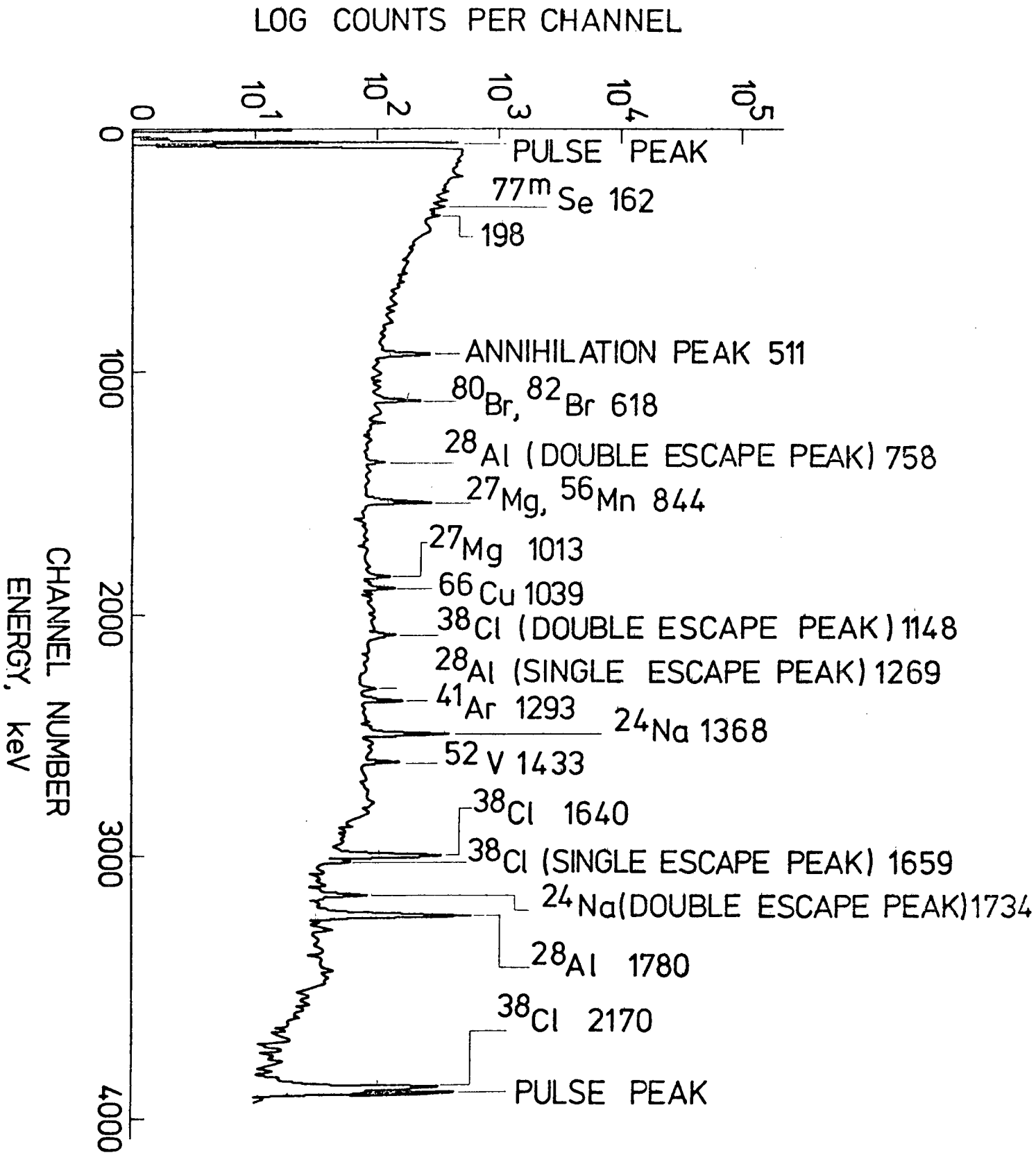


Figure 65: Gamma spectrum of a liver sample after ninety seconds of irradiation and twenty seconds of decay time

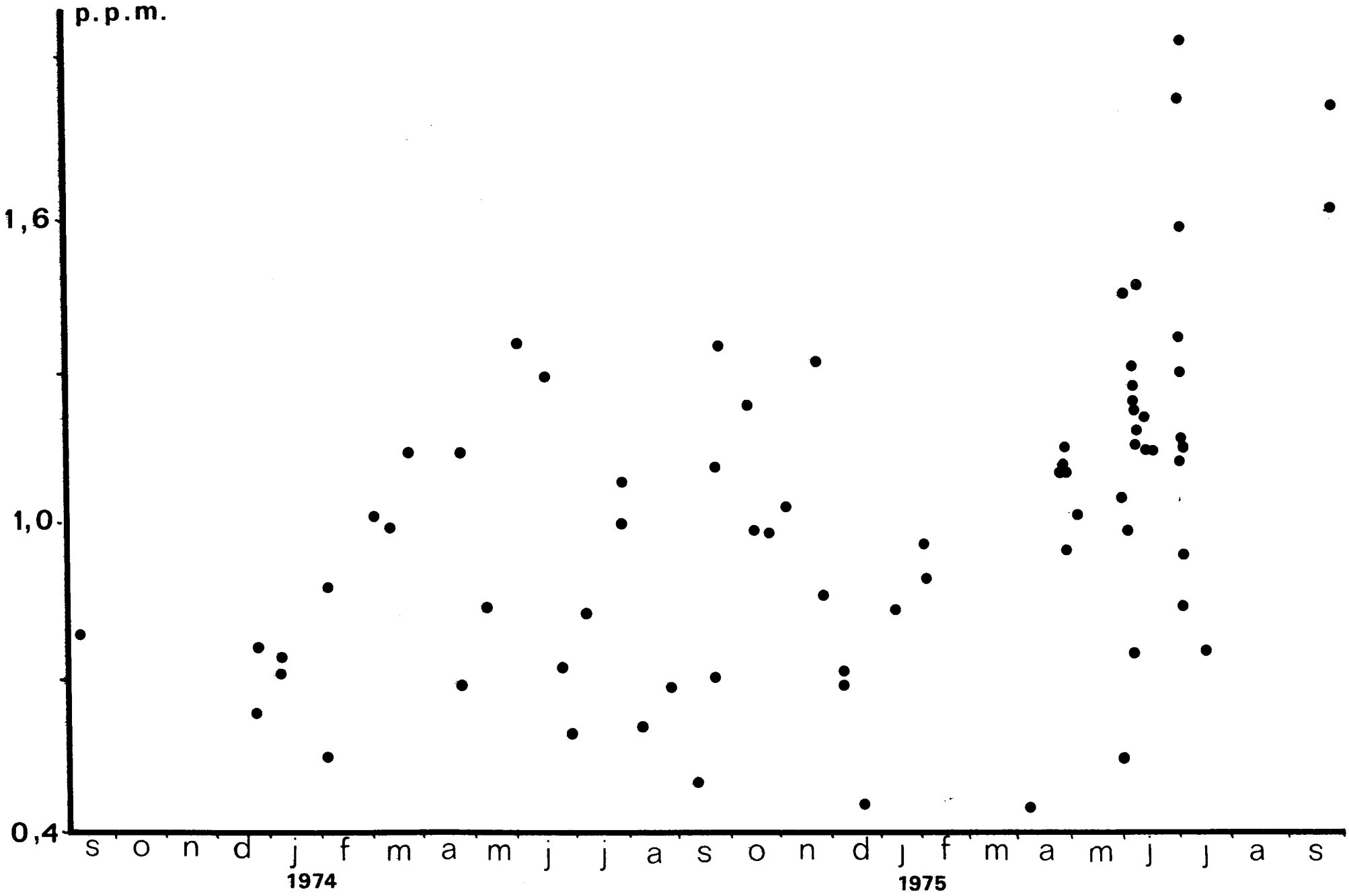


Figure 66: Liver selenium content of seventy-four animals over twenty-five months in all sample areas.

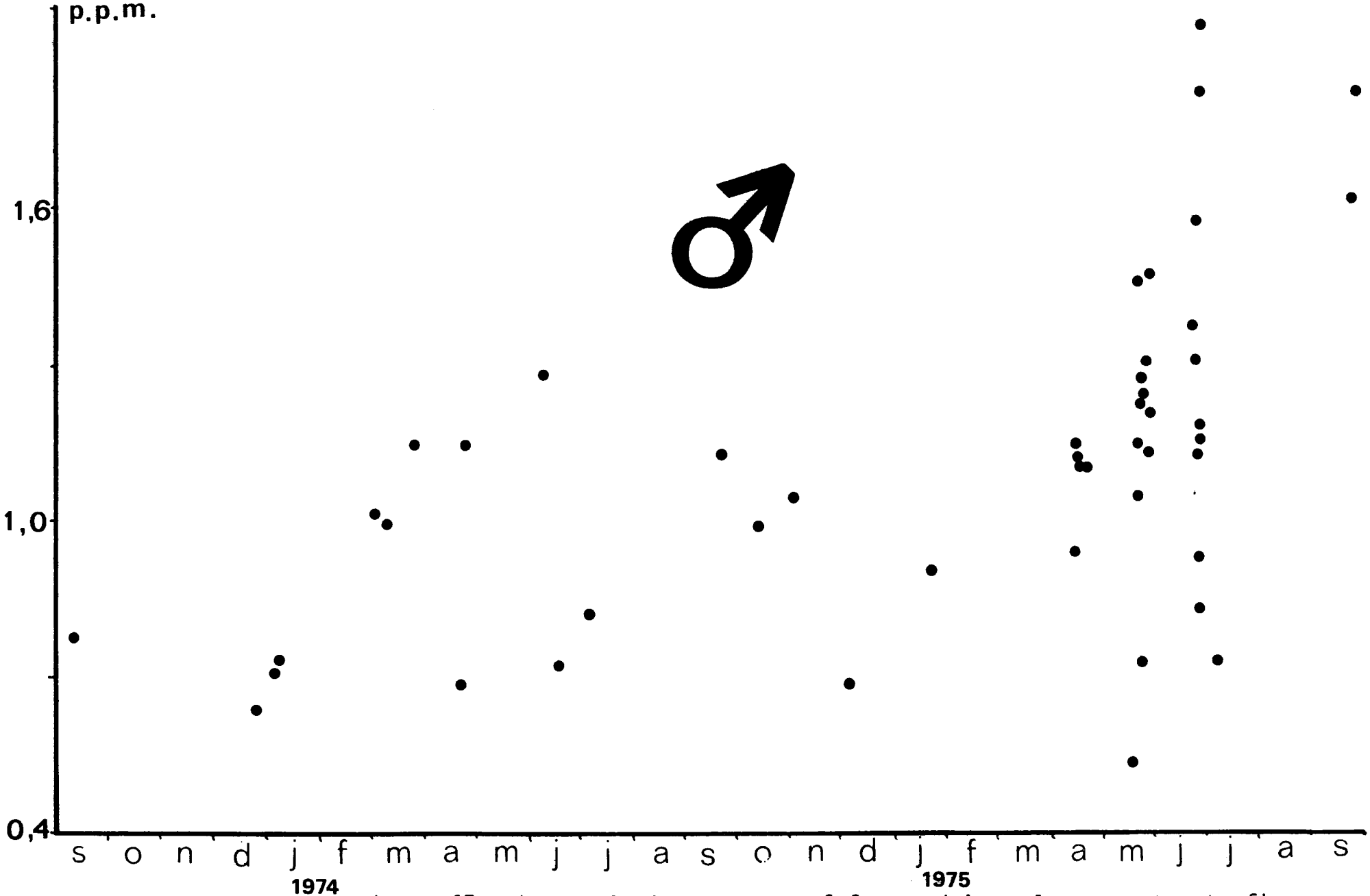


Figure 67: Liver selenium content of forty-eight males over twenty-five months regardless of species or area.

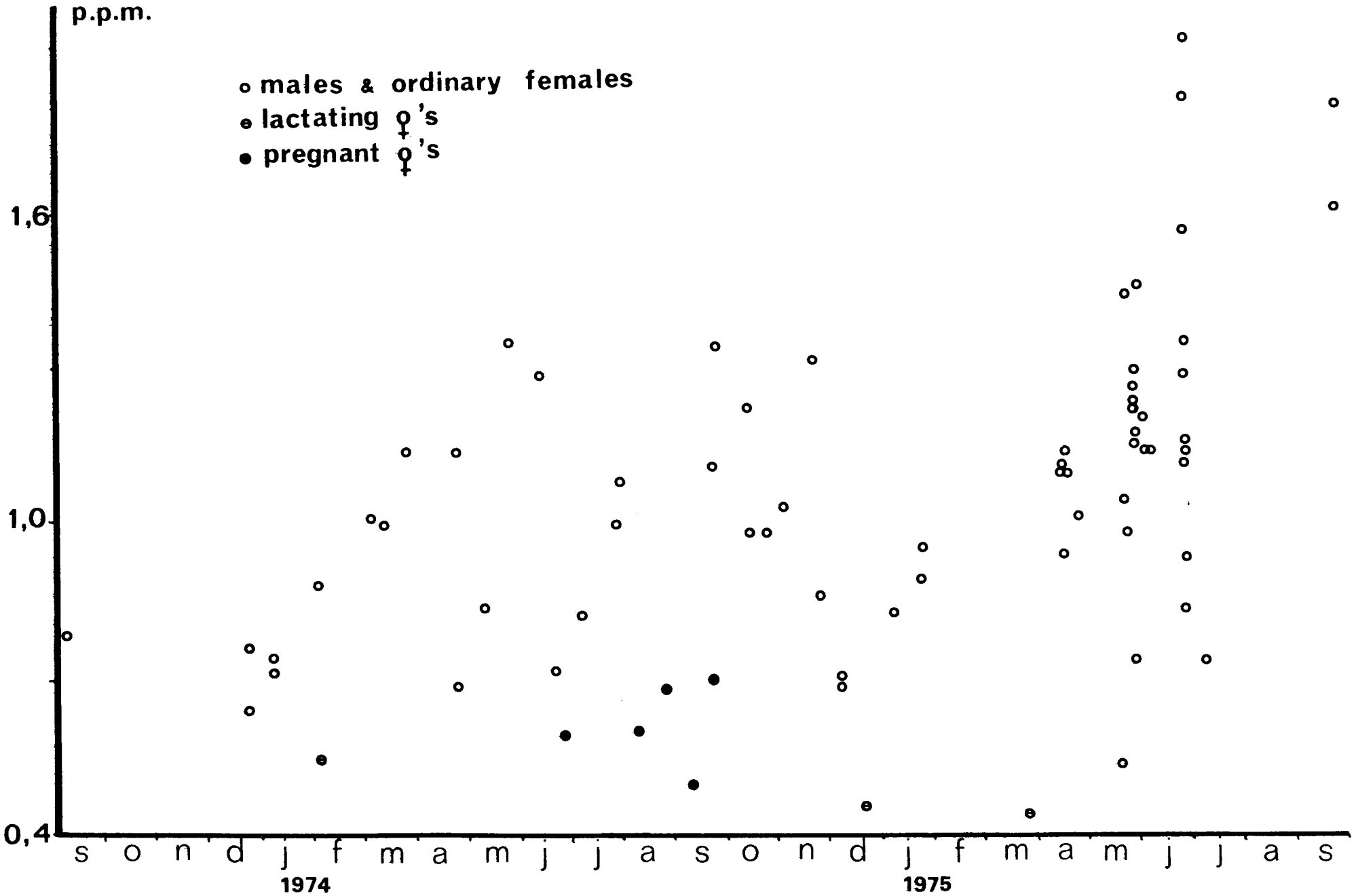


Figure 68: Liver selenium values of seventy-four animals over twenty-five months showing pregnant and lactating females apart from main group.

percent between the extreme months.

It is noteworthy that lactating females are approximately 100 nanograms or more below the other levels. There is a lactating warthog in February showing the lowest selenium values of all. It is well known that pregnant females are more susceptible to capture myopathy (e.g. three pregnant females were the only animals that died in a recent consignment of 15 blesbok to Bronkhorstspruit and the only other animal showing symptoms was a female) and are often the only ones to succumb. The possible relationship of lactation or pregnancy and selenium depletion may well bear investigation. Piglets are known to receive a store of iron in the liver from the sow during the gestation period, to tide them over the time when they receive only milk (Calhoun and Smith 1958). A similar pattern probably obtains with other trace elements. It is possible and even likely that females which are pregnant or lactating during the months of selenium shortage may well aggravate their tissue shortage by channelling selenium into their young either by storage in the foetus, through lactation or both.

Spearman's coefficient of rank correlation was applied only to the collective data and the male animal values, as they were the only sets of data stretching over the minimum period of two years (in this case 25 months) allowed for this type of analysis. From this set of data, statistically significant results were also not obtained, but for

the sake of comparison the r_s (correlation coefficient) was calculated in both cases. The values were grouped into two sets, each representing corresponding values in corresponding months of the sample year. They were then ranked accordingly and the results were as follows:

The composite group: $r_s = 0,05$

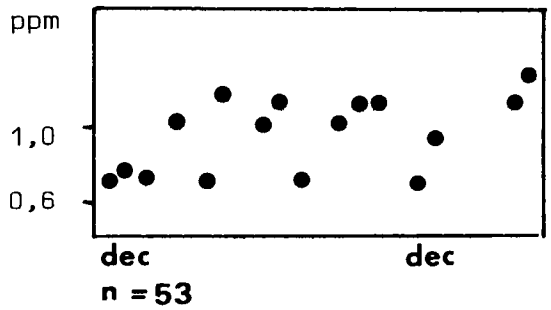
The all males group: $r_s = 0,28$

Although neither result was statistically significant, there is indication that a seasonal variation may not hold good for composite results, and should be broken down to separate sex, and if further amounts of samples were available over a longer period, perhaps further breakdown may be indicated, i.e. into area or species. The difference in results of the composite group and the all males group suggests that further work should be done along these lines. The results relating to the other groups are given in the graphs plotted in Fig. 69 .

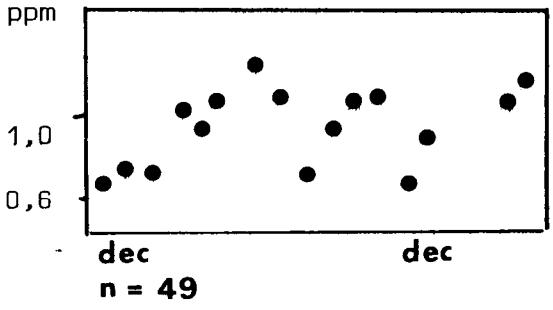
DISCUSSION

THE results of the analyses show remarkable conformity considering that the samples were derived from a number of different species and several geographical locations. The first low levels occur in September, dropping gradually until December after which the values commence to rise, reaching peak values in April and May. The levels do not follow the same pattern as the rains. The first substantial rains fell in September (Fig. 2 to 6 in Chapter One)

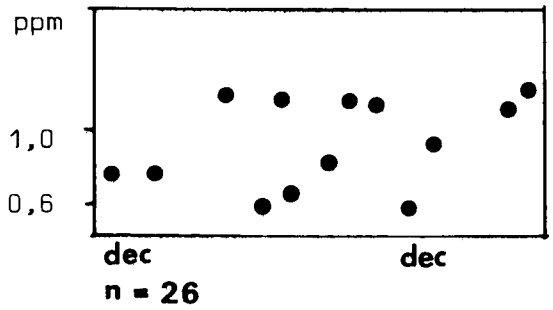
monthly averages



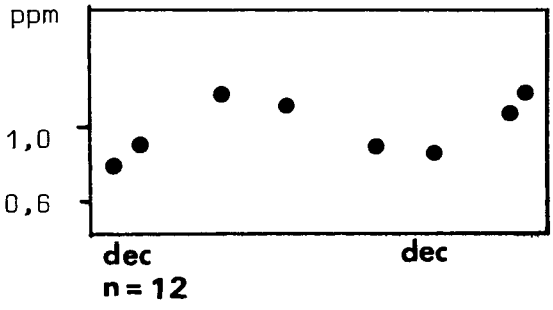
Umfolozi - all



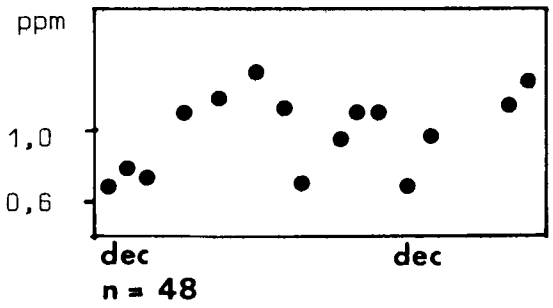
warthog - all



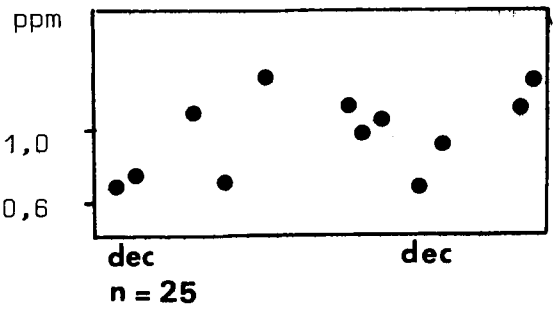
females - all



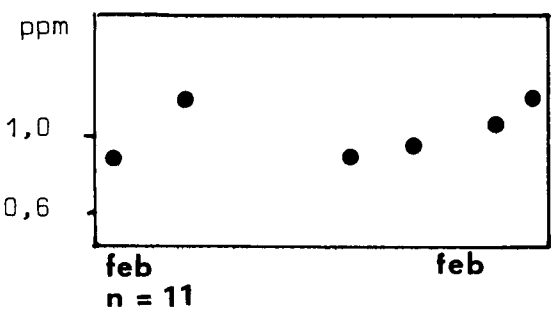
ordinary females



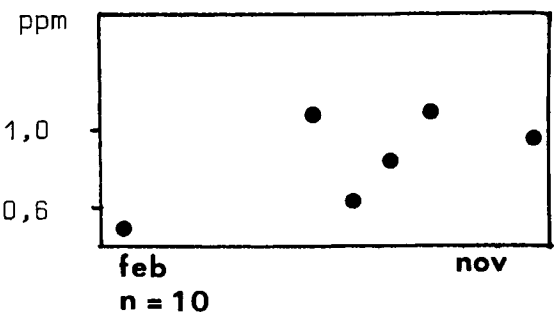
Umfolozi warthog



Umfolozi warthog males



Umfolozi warthog - ordinary females



Umfolozi female warthog - pregnant or lactating

Figure 69: Grouped results of liver selenium according to areas, sex and species.

and another peak in selenium values was evident in October and November.

There is another low period in August and September, but this may be an artifact caused by scatter of results at this season. Other samples collected at this time have yet to be processed, and the trend may eventually be more exactly determined.

As increased susceptibility to capture myopathy in wild animals during the latter part of the dry season is generally accepted, although factors such as low forage protein value are undoubtedly a major cause, the low levels of selenium during this time of the year are likely to be a contributing factor.

OTHER TRACE ELEMENTS

SIXTEEN other trace elements were analysed over eight and fifteen months. Figure 70 shows the levels, over 15 months for chlorine, cobalt, magnesium, sodium and zinc. The levels of these elements show remarkable conformity with the main peaks at approximately the same time in July. The graph illustrates the problem that may arise if single or only a few specimens are analysed to give indications of trace elements. If we can assume that the peaks in differing areas coincide (as they appear to do) considerable error may result by comparing samples from one area,

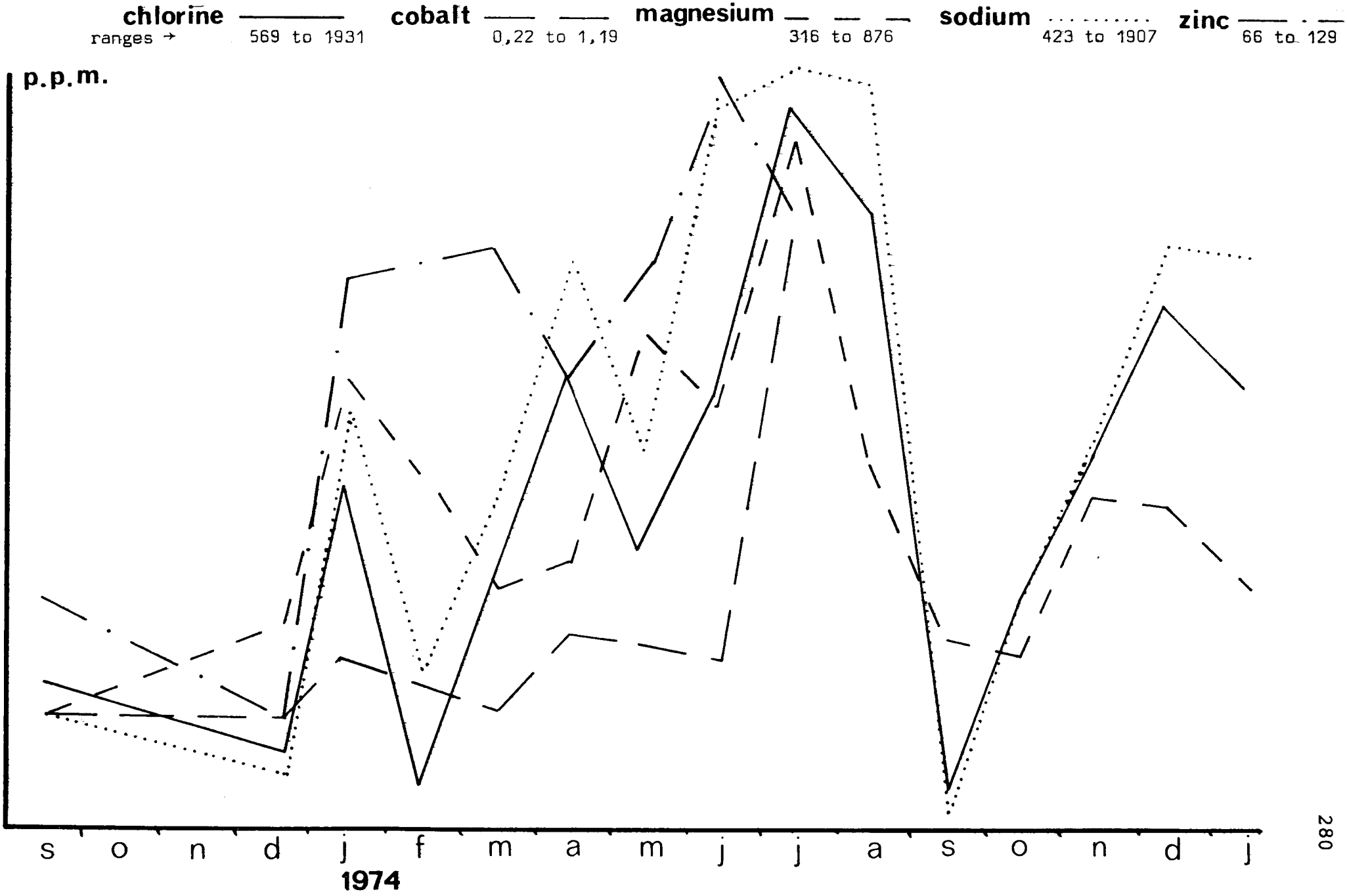


Figure 70: Composite graph of various species' liver content of five elements over fifteen months.

say in June, and from another in August; the difference for a number of elements between these months amounting to or exceeding 200 percent in most cases.

The five elements mentioned above were analysed statistically for correlation. These results were from 15 months, although cobalt and zinc were determined only over eight months. The following correlations resulted, with their levels of significance (all tests were two-sided).

chlorine with cobalt: $r = 0,8578^{***}$ $(n = 8)$

chlorine with magnesium: $r = 0,6044^{**}$ $(n = 15)$

chlorine with sodium: $r = 0,9540^{***}$ $(n = 15)$

chlorine with zinc: $r = 0,6799^{*}$ $(n = 8)$

cobalt with magnesium: $r = 0,7974^{**}$ $(n = 8)$

cobalt with sodium: $r = 0,6526^{*}$ $(n = 8)$

cobalt with zinc: $r = 0,3518$ not significant $(n = 8)$

magnesium with sodium: $r = 0,6263^{**}$ $(n = 15)$

magnesium with zinc: $r = 0,6570^{*}$ $(n = 8)$

sodium with zinc: $r = 0,8399^{***}$ $(n = 8)$

In these comparisons, wherever one element had only eight months' values, then from the element being compared, only eight months' values were taken, regardless of number of months actually analysed.

The linear regression for three of the comparisons was calculated as follows:

chlorine with sodium: $y' = 1,1638x - 146,354$

cobalt with magnesium: $y' = 456,129x + 368,839$

sodium with zinc: $y' = 0,0342x + 60$

The most significant correlations were (in order of significance) chlorine with sodium, chlorine with cobalt, sodium with zinc, and cobalt with magnesium.

Although the time period over which the samples were analysed is not long enough to give any statistical indication of seasonal variation or seasonal index, the correlation between certain trace elements is interesting to note, especially since three of the comparisons appear to be highly significant.

The remaining eleven elements which upon observation appeared not to have too close a correlation, are depicted in Fig. 71 and 72. Copper appears to follow a similar pattern to selenium, although statistical correlations have not been made. Most elements appear, as does selenium, to have two peaks during the year.

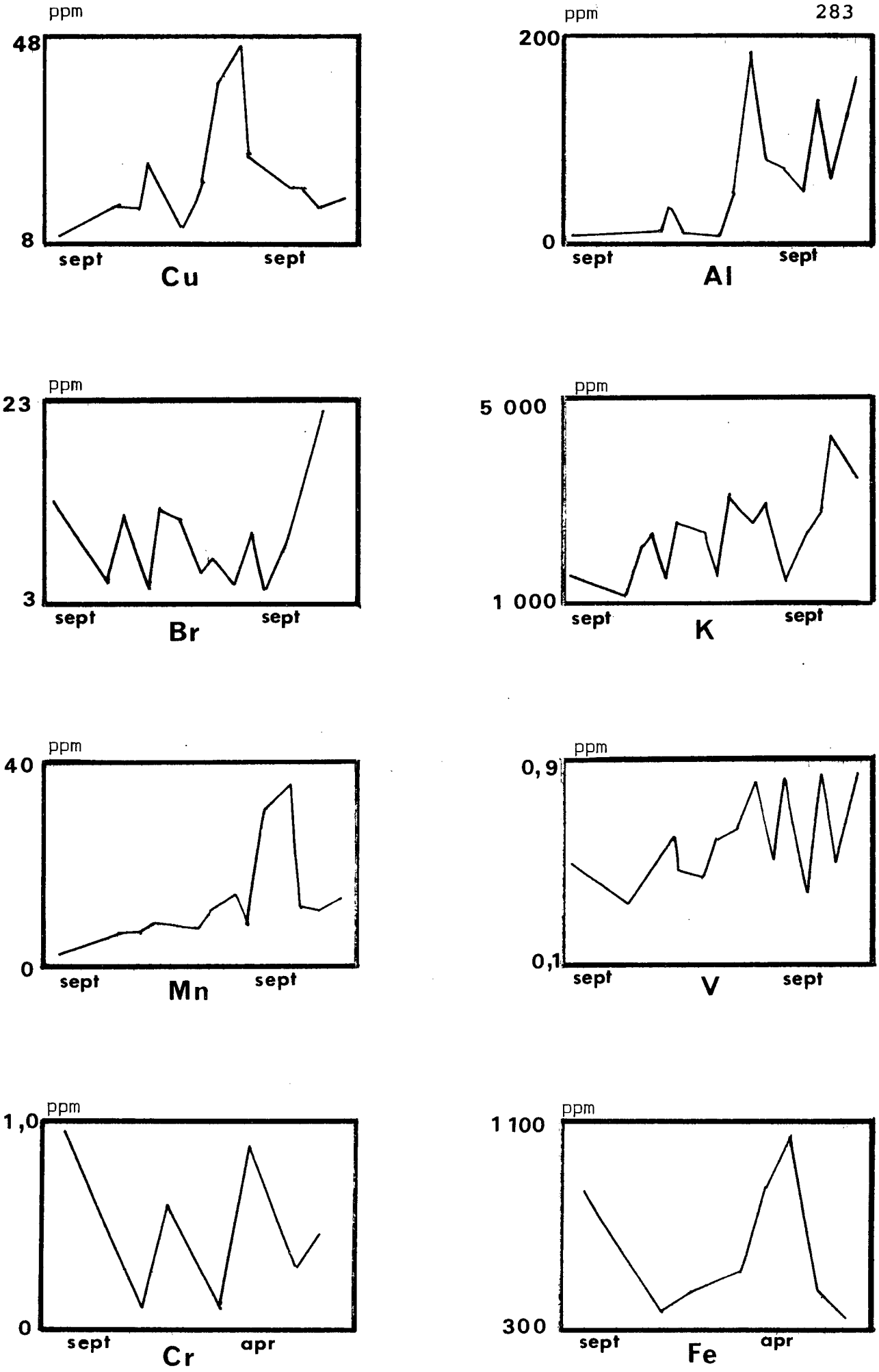
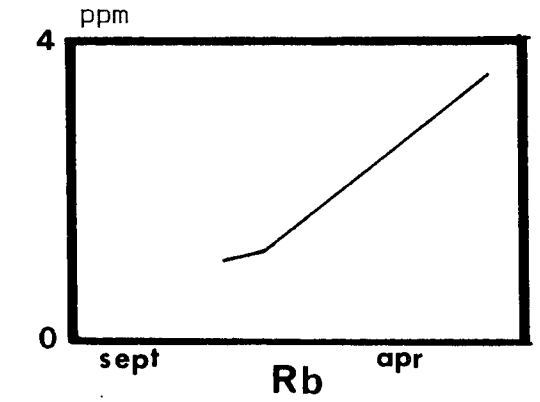
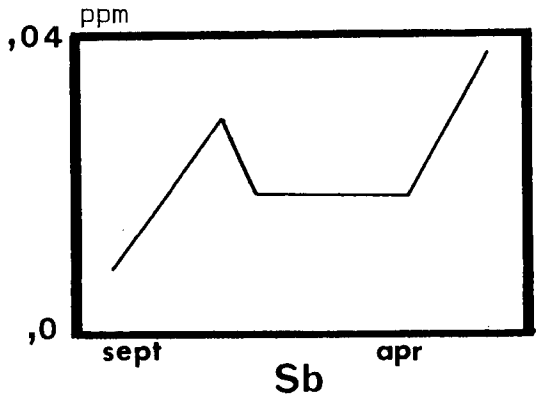


Figure 71: Liver content of various elements.





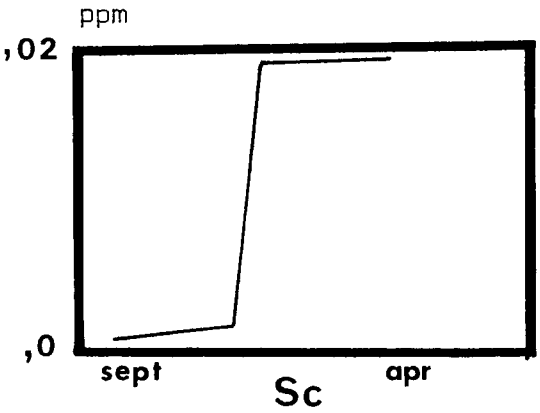


Figure 72: Liver content of various elements.

CHAPTER FIFTEEN

THERAPY

BICARBONATE

INTRODUCTION

TREATMENT of the acute form of capture stress by bicarbonate infusion has been largely dealt with in Chapter Four. This section deals mainly with the clinical effects.

MATERIALS AND METHODS

SIX zebra were originally used in this experimental series. These animals were subjected to maximum forced exercise in the Kruger National Park and caught and restrained as described in Chapter Two. Infusion was administered at the rate of 1 000 m-equiv./l bicarbonate in physiological saline or in a balanced solution of ions per 250 kg body weight.

RESULTS AND DISCUSSION

THE most notable effect of the infusion was an immediate improvement in condition. The animals became visibly more alert, and often started to struggle from being in a near-comatose state. On auscultation, the change in the heart sounds appeared remarkable. The heart beat improved in

strength. Prior to infusion, heart sounds were muffled and indistinct. On infusion these became crisp, louder and in many cases audible to bystanders, the apex beat becoming visible on the body surface. In a similar way the breathing became less distressed and slowed down after an early increase in rate.

The extent of the improvement was more remarkable than the changes in the pulse and respiratory counts, although these also were significant. The pulse rate, taken immediately after the first infusion, fell from an average of 244 to 144 beats per minute. Similarly respiration fell from an average in all surviving animals from 65 to 56 per minute, although there had been an increase in respiratory rate prior to the time of infusion (see Fig. 28).

The three animals that were not treated deteriorated progressively, and died at 30 minutes, 9 hours and 12 hours respectively after capture. None of the treated animals died.

In many cases the hydrogen ion concentration of the blood increased over the period between capture and infusion, in spite of complete restraint and 'unhampered' breathing. The extent of the low pH after the forced exercise was remarkable. In the surviving animals, after the first chase, the average pH on capture was 6,78 falling to 6,67 before infusion (Table 7).

After infusion of 500 m-equiv. the pH rose to an average figure of 7,13 and after 1 000 m-equiv. to an average of 7,28 (Fig. 40).

Figure 73 depicts the direct pH measured and that of the blood equilibrated with 4 percent and 8 percent CO_2 and O_2. It may be seen that the lines depicting the pH of the blood samples on capture and before infusion lie far to the left of the normal limits of the Siggaard-Andersen Curve Nomogram (line XY) necessitating an extension (XZ). After infusion there was a shift back into normal ranges.

As previously explained (Chapter Thirteen) the bicarbonate therapy has other effects besides returning the pH to a physiological range. The potassium of the plasma is reduced by approximately 0,6 m-equiv. per 0,1 rise in pH units enabling the potassium to re-enter the cells. There is also an immediate dilution of potassium in the plasma by a factor of approximately 10 percent through mechanical dilution while additional calcium ions are supplied if the bicarbonate is dissolved in 'Normosol' (balanced ion) solution. The extra fluid counteracts dehydration and promotes kidney action, the elimination of blood and muscle pigments besides counteracting the tendency for these to be precipitated by acid conditions.

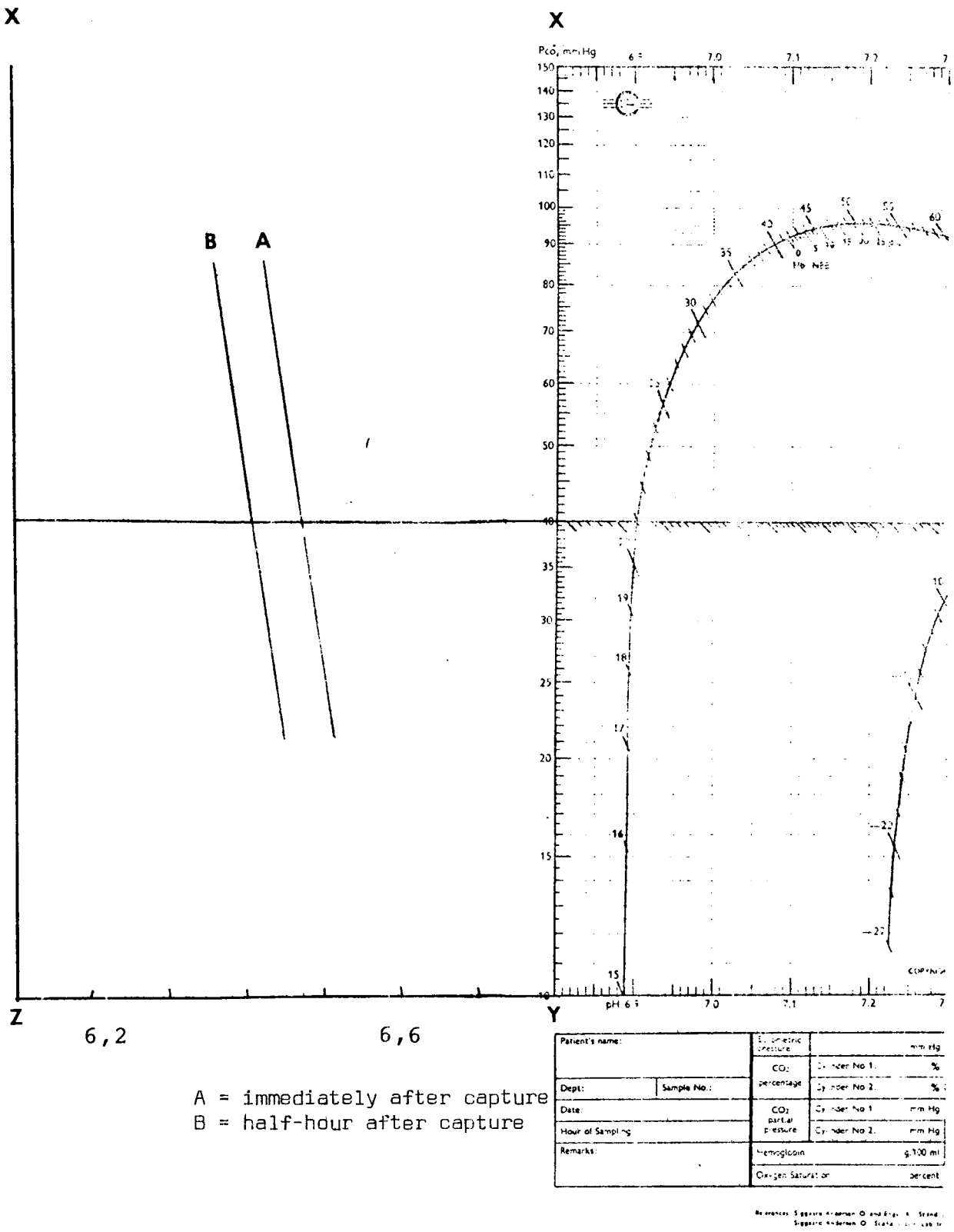


Figure 73: Siggaard-Andersen curve nomogram extended to show acid-base values in zebra immediately after capture and half-an-hour later.

SELENIUM AND VITAMIN E THERAPY

Materials And Methods

NINE tsessebe were kept in enclosures at Percy Fyfe Nature Reserve. Four of these animals were given an injection of 'Bo-se', a preparation of selenium and 'Tocopherol' designed for the prevention and treatment of 'selenium-Tocopherol-deficiency' syndrome in calves and sheep. It contains sodium selenite (equivalent to selenium 1 mg) 2,19 mg, and vitamin E (as *d*-alpha tocopherol acetate) 68 I.U. The same animals were given a further injection immediately after forced locomotory exercise. Exercise was conducted on an exercise track (Chapter Two).

Results

NOTABLE differences in blood levels between the treated and untreated groups of tsessebe with regard to LDH and CPK were observed (Fig. 74).

An initial lowering of blood enzymes subsequent to the first injection of 'Bo-se', was seen to occur before the second injection three weeks later. No exercise was given during this period, the animals remaining in their enclosures.

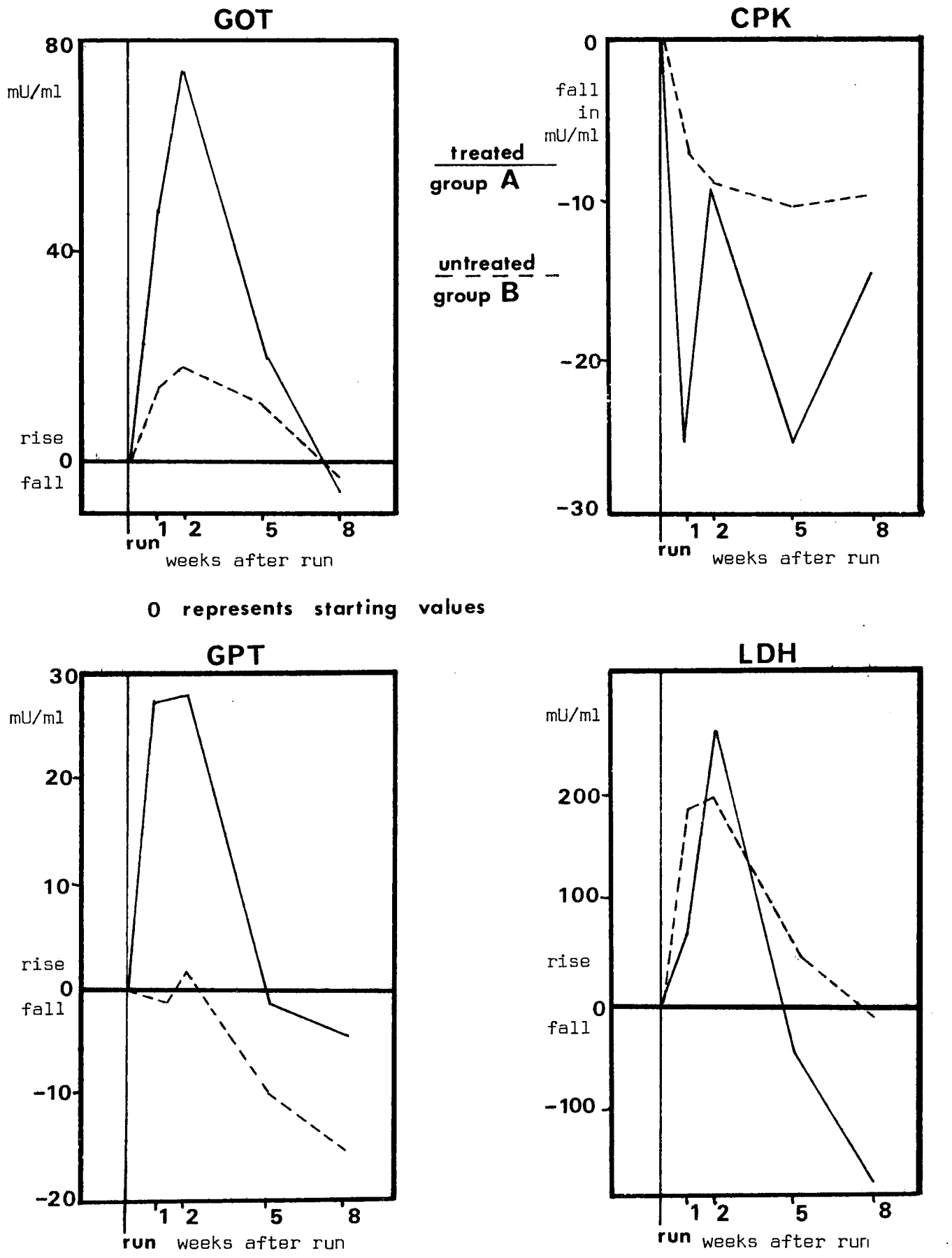


Figure 74: Differences in enzyme levels between treated and untreated tsessebe.

LACTATE DEHYDROGENASE

THE extent of the drop in LDH values in all four treated animals (Group A) ranges from 248 to 907 mU/ml with an average of 517 mU/ml. The untreated group (B) showed a fall in LDH in the same period of 151 mU/ml, i.e. a difference of 366 mU/ml.

At four weeks, that is one week after forced locomotory exercise over 2 km, the LDH in Group A showed an average rise of 60 mU/ml ranging from a drop in one animal of 66 to a rise of 124 mU/ml. Group B over the same period showed a rise averaging 187 mU/ml with a range of rise of 67 to 411 mU/ml.

CREATININE PHOSPHOKINASE

ESTIMATION of CPK levels provided a similar picture to that of LDH although the results were less clear cut. Group A after the first injection of 'Bo-se' showed an average drop of 311 with a range of 71 to 598 mU/ml. After exercise a further drop occurred in the blood level of CPK of 25, ranging from 23 to 31 mU/ml.

In the untreated animals (Group B) the results were distorted by one animal coming down to near normal levels from a very high reading, dropping 283 mU/ml. Excluding this animal, there was an average rise of 6 mU/ml. This was fairly constant as three animals showed an identical rise of 8 mU/ml and one showed no change.

After exercise, Group B showed a scatter of results with some animals showing a rise and others showing a fall so that the results are difficult to interpret in a meaningful way.

Glutamic Oxaloacetic Transaminase

The levels of GOT in Group A estimated after the first injection showed an average fall of 16 ranging from a rise of 6 to a fall of 64 mU/ml. There was a rise after exercise in this group averaging 48 with a range of 13 to 144 mU/ml. Group B showed an average rise of 12 after chasing, with a scatter of results after the first injection, with all animals except one showing a drop in GOT values.

Glutamic Pyruvic Transaminase

The GPT levels in Group A showed a drop averaging 41 after the first 'Bo-se' injection ranging from 0 to 76 mU/ml. This rose, however, after exercise to an average figure of 28 ranging from 4 to 55 mU/ml. Group B showed a smaller fall after the first injection of 24 mU/ml, but here again the results are difficult to interpret owing to one reading of 116 mU/ml. Otherwise there was a rise in GPT in this group compared to the fall as shown in Group A. After exercise the animals were equally divided with two showing a rise and two a fall in GPT, and one remaining static.

Statistical comparisons were made between values immediately after exercise and subsequent values over a period of weeks, using both the *t*-test for paired observations and

the Wilcoxon matched pairs signed rank test. The treated groups were compared to the untreated groups in this way and the results were as follows:

GOT: t_4 = -1,87 not significant at 10 percent (n = 5)

T(P,N) = 9, T(N,N) = 1 significant at 10 to 25 percent (n = 4)

GPT: t_4 = -2,739* (n = 5)

T(P,N) = 10, T(N,N) = 0* (n = 4)

CPK: t_4 = 2,030 not significant at 10 percent (n = 5)

T(P,N) = 0, T(N,N) = 10* (n = 4)

LDH: t_4 = 1,552 not significant at 10 percent (n = 5)

T(P,N) = 1, T(N,N) = 9 significant at 10 to 25 percent (n = 4)

On the t-test, only the GPT seems to have been significantly affected by the 'Bo-se' therapy, but negatively so. According to the Wilcoxon test, only CPK and LDH drop significantly as a result of the treatment and this is reflected in Fig. 74.

Discussion

THE results of 'Bo-se' injection into tsessebe are not clear cut. This may be due to a number of factors including the small number of animals available, the difference in length of captivity, and therefore of tameness. Also their differences in temperament, some animals exhibiting a greater reaction to disturbance than others.

There did, however, appear to be a therapeutic effect from

'Bo-se' injection on LDH which showed a precipitous drop of 517 mU/ml as compared to 151 mU/ml in the untreated animals. A slight rise occurred after exercise of 60 mU/ml as compared to a rise in the untreated group of 187 mU/ml.

The levels of CPK also showed a rapid decline after 'Bo-se' injection of 311 mU/ml and a further drop of 25 after exercise, and a subsequent injection.

A major problem presented here is the stressed condition of all animals prior to exercise. The exercise itself showed no more than moderate stress so that there was no marked rise of enzyme levels in the untreated animals after exercise except possibly for LDH.

Two major differences appear to exist between the results of exercise on the track during this experiment, and that of chasing in the wild. These may be due to several factors including greater efforts on the part of the latter to escape from the pursuing vehicle, use of a greater porportion of the musculature, and fear in the untamed animal. Also the speeds obtained in the track were lower during this series of experiments than attained under bush conditions. The animals used were fed artificially on a diet largely of fresh lucerne and therefore the probability of a lack of selenium and/or vitamin E in the diet is small and far less than that of wild animals suffering deficiency at certain times of the year. The administration of 'Bo-se'

three weeks before exercise is hardly a practicable basis for treatment of captured animals, but did enable some observations to be made on the possible role of adequate selenium and vitamin E levels in preventing stress or capture myopathy.

Clinically, 'Bo-se' has been reported as being ineffective in the treatment of tsessebe for capture myopathy (Young 1972). This may be due to several causes such as delay in treatment and therefore the treatment of established capture myopathy, as also attempted treatment of severe cases. The determination of the impact of therapeutically effective substances on the enzyme levels which are known to indicate capture stress probably provides a more sensitive criterion of putative therapeutic activity.

RATIONALE OF PHENOXYBENZAMINE THERAPY

Introduction

The blockade of alpha receptors with phenoxybenzamine hydrochloride has been used as an attempted therapy for the acute form of capture myopathy. There was a further indication that where the acute form is relieved, the sub-acute and chronic form are unlikely to develop. Phenoxybenzamine was used at a low concentration of approximately 20 mg per litre and given at a slow rate of infusion of one litre over one hour. The object of this treatment was to alleviate

the sympathetic vascular hypertonus that is almost certainly present in all newly captured animals. The principal effectiveness of phenoxybenzamine at this range of concentration is on the post-synaptic alpha receptors rather than the pre-synaptic alpha receptors that regulate transmittor-release (see below).

Previous Results

There is evidence that at least part of the reactive state of the animal after capture may be ascribed to intense vasoconstriction. This is turn results in decrease of the circulatory blood volume, and increase in metabolites in parts of the circulatory network, and probable damage to the kidney and other parenchymatous organs.

At concentrations of $2,9 \times 10^{-7}$m the neuronal uptake of noradrenaline is inhibited by about 20 percent (Cubeddu, Langer and Weiner 1974). Higher concentrations were employed by others (Langer 1970, Langer and Vogt 1971, Bennett 1973), but without a comparable increase in blockade of end organs such as the spleen to nerve stimulation or reduced response to exogenous noradrenaline.

It has been found (Dubocovich and Langer 1974) that phenoxybenzamine was more potent in blocking the post-synaptic alpha receptors that mediate the vascular responses to both exogenous and endogenously released noradrenaline than in blocking the pre-synaptic alpha receptors which regulate transmitter release during nerve stimulation. The level of

noradrenaline in the circulation is therefore not reduced, but only the circulatory effects. At the same time, at low concentrations the actual concentration of transmitter is not increased. As was pointed out by the last mentioned authors, the concentration of phenoxybenzamine needed for the blockade of the pre-synaptic alpha receptors that regulate transmitter release is 30 times higher than that required for the blockade of the receptors of the effector organs. It is possible, therefore, that the pre- and the post-synaptic alpha receptors are not the same; neither may the affinity of the two types of receptors for the blocking agent or for the agonist noradrenaline be identical. It may be noted also that a spare receptor population has been postulated (Waud 1968, Langer and Trendelenburg 1968) to account for this phenomenon. Since our object was to reduce vascular resistance the lower range of phenoxybenzamine has been used.

Oliguria due to renal tubular blockage by myoglobin or haemoglobin derivation should therefore be ameliorated by phenoxybenzamine hydrochloride therapy. Such therapy, using the very low concentrations of phenoxybenzamine is therefore indicated where there is discolouration of the plasma whether this has been caused by excessive and forced exercise, by massive injury such as bruising, or from metabolic disturbance such as azoturea - paralytic myoglobinurea.

The phenoxybenzamine hydrochloride should be administered

prophylactically rather than curatively within the context of capture stress. Once shock has set in or anuria is an accomplished fact, the treatment, if any, is complex and the chances of recovery greatly reduced. Also, it would then be necessary to administer massive infusions of blood volume expanders, such as 'Low Molecular Weight Dextran' or blood plasma to prevent shock.

The advent of shock may be gauged by clinical signs. These are: Cold extremities, sweating in some animals, weak rapid pulse, low pulse pressure, collapsed peripheral veins, sluggish peripheral circulation, and discoloured mucous membranes. Successful therapy can be gauged from a reversal of these symptoms, the pulse becomes full and the extremities warm and dry.

PHARMACOLOGY

THE generic name is phenoxybenzamine hydrochloride and the chemical name is N-(2-chloroethyl)-N-(1-methyl-2-phenoxy-ethyl)benzamine hydrochloride. The molecular weight is 340,31 and the empirical formula is given as $C_{18} H_{22} Cl NO.HCl$.

RESULTS

WHILE the available cases on which this type of therapy could be tested are as yet few, certain indications as to its effectiveness are evident.

The blood picture before, during and after infusion shows an improved perfusion of tissues. This improved perfusion is similar to that obtained with the bicarbonate infusion in acidotic animals. Experiments on sable antelope indicated that the infusion of low levels of phenoxybenzamine hydrochloride reduced considerably the level of the pulmonary arterial pressure (see Plate 9). The results of phenoxybenzamine on the levels of blood metabolites are given in Chapter Eleven.

It seems likely, therefore, that the combination of the two forms of therapy should show improved effectiveness in reducing vasoconstriction irrespective of whether the prime cause of increased vascular resistance and reduced tissue perfusion has its origin in low pH or in adrenergic hypertonus.

The results so far indicate that once the blood flow has been re-established the greatest danger to the animal has been surmounted. This may be especially relevant in relation to reduction of muscle damage by high local concentrations of lactic acid, and the aleviation of blockage of the tubules of the kidney; it has been pointed out elsewhere (Chapter Sixteen) that accumulation of blood and muscle pigments in the kidney under certain conditions such as intense vasoconstriction, increases the liability to blockage and resultant anuria.

Enzyme levels over the ensuing weeks compared to that of

control animals also suggest that damage to other parenchymatous organs such as the liver may also be reduced as a result of prompt infusion therapy with phenoxybenzamine.

The length of time that this treatment is effective is not yet known. It is likely, however, that once excessive sympathetic tonus of blood vessels is released, further avoidance of fear and excitement and possibly the judicious use of tranquillisers should reduce the necessity for further treatment.

DISCUSSION

PHENOXYBENZAMINE hydrochloride is a persistent alpha-blocking agent which blocks the excitatory response of smooth muscle to both noradrenaline and adrenaline. It leaves the inhibitory responses intact. It inhibits the excitatory response of exocrine glands, but not that of the myocardium to adrenaline and related compounds.

Spinal reflexes and the thoracicolumbar sympathetic outflow are not affected. It does not block adrenergic mediators, or cause sympathetic ganglionic blockade. The reaction of smooth muscle to non-sympathetic stimuli remain unaltered.

Although the excitatory response of the myocardium remains unaltered, a fall in blood pressure resulting from phenoxybenzamine hydrochloride must not be countered with adrenaline,

as a further fall in blood pressure may result due to adrenaline reversal. No marked fall in blood pressure should result from the administration of phenoxybenzamine hydrochloride except in cases of shock. In that case, copious fluid therapy should be instituted. Severe hypotension that does not respond to fluid therapy should be treated with vasopressive agents.

Under normovolaemic conditions, the injection of phenoxybenzamine hydrochloride causes little or no change in the blood pressure. It does, however, increase the blood flow in the peripheral circulation, in organs such as the kidney, and increases the cardiac output; i.e. in all cases where there is an adequate circulating volume.

The value of phenoxybenzamine hydrochloride is for cases where there is persistent vasoconstriction whether in the peripheral circulation, the lung or the kidney. Such a constriction may be due to inadequate circulatory volume. In many cases, however, and in most cases involving capture stress, the blood volume is adequate, and the cardiac output, at least initially, is sufficient. In spite of this, there appears to be a vasoconstriction, particularly a renal vasoconstriction, due to reflex sympathetic nervous activity or sympathetic hypertonus. As a result, the urine output is apparently decreased. In such cases, phenoxybenzamine hydrochloride may be effective in inducing an adequate renal response.

PARENTROVITE AS A SUPPORTIVE THERAPY

INTRODUCTION

COMPLEX derivatives of the B group of vitamins form co-enzymes. In these, the vitamins build up into various nucleotide-like structures. These act as coenzymes and play a very important part in the process of biological oxidation. Different enzyme systems may be derived from one and the same coenzyme that can react with different apoenzymes. In this way thiamine is phosphorylated in the tissues to yield thiaminepyrophosphate (TPP, cocarboxylase), which can act as coenzyme with a different apoenzyme. In thiamine deficiency, pyruvic and lactic acid accumulate in the body tissues and fluids as they cannot be metabolised. Without TPP, an essential component of the citric acid cycle, the latter, or tricarboxylic acid cycle is unable to function.

It may be pertinent to note that diets high in carbohydrates and low in fats are conducive to thiamine deficiency. Fats are unlikely to be available as a dietary source on many types of grazing.

Thiamine has been recommended as treatment for paralytic myoglobinurea (azoturea) in horses and mules, together with supportive therapy (Bauch 1945, Stroup 1945).

'Parentrovite' is composed of thiamine hydrochloride BP

35 mg; riboflavine 0,5 mg; pyridoxine hydrochloride BP. 7 mg, and nicotinamide 23 mg, for each millilitre, and was administered at a rate of 20 ml for each animal, followed by the same dose three days later at the first repeat sampling, each dose given intramuscularly. Three animals were treated and three were used as controls.

Materials And Methods

Six tsessebe bulls were used, weighing 93 to 123 kg, with a mean weight of 105,5 kg. They were subjected to forced exercise on a specially constructed exercise track situated at Percy Fyfe as describe in Chapter Two. The animals were stressed individually at a mean speed averaging 22,2 km/h for a distance of exactly two kilometres, the range of average speeds being from 17,27 km/h to 25,7 km/h. The procedure followed that described in Chapter Two.

Measurements were taken together with blood samples for enzyme and blood metabolite determinations also described in Chapter Two.

Results

Creatine Phosphokinase

The difference between the treated and untreated groups in respect of CPK was small (i.e. 140 and 160 mU/ml) and this figure in fact reflects the starting difference between the two groups. The time taken for all animals to reach

normal levels was almost identical, although there was a sharper fall in the treated group (Fig. 75).

Glutamic Pyruvic Transaminase

The reaction to 'Parentrovite' as shown by GPT levels indicate no advantage from the therapy (Fig. 76). In fact the GPT levels in the treated animals were slightly higher than those in the untreated group. However, none of the animals showed marked rises in GPT, the maximum level being 65 mU/ml, so that the difference is probably of no significance.

Glutamic Oxaloacetic Transaminase

The starting points of GOT levels were almost identical while the peaks showed a marked difference, i.e. from 155 in the treated group to 195 mU/ml in the untreated (Fig. 77). The recovery of the treated group was somewhat slower than than of the untreated group. There seems to be a considerable amount of discrepancy in the reactions in respect of GOT to this treatment, and this is reflected in the ranges (Table 20).

Lactate Dehydrogenase

The results with regard to LDH appear to be significant from the graph, although they are not statistically significant. High mean levels exceeding 900 mU/ml in the untreated group compare with 416 mU/ml in the treated group one day after exercise, the latter commencing at a higher

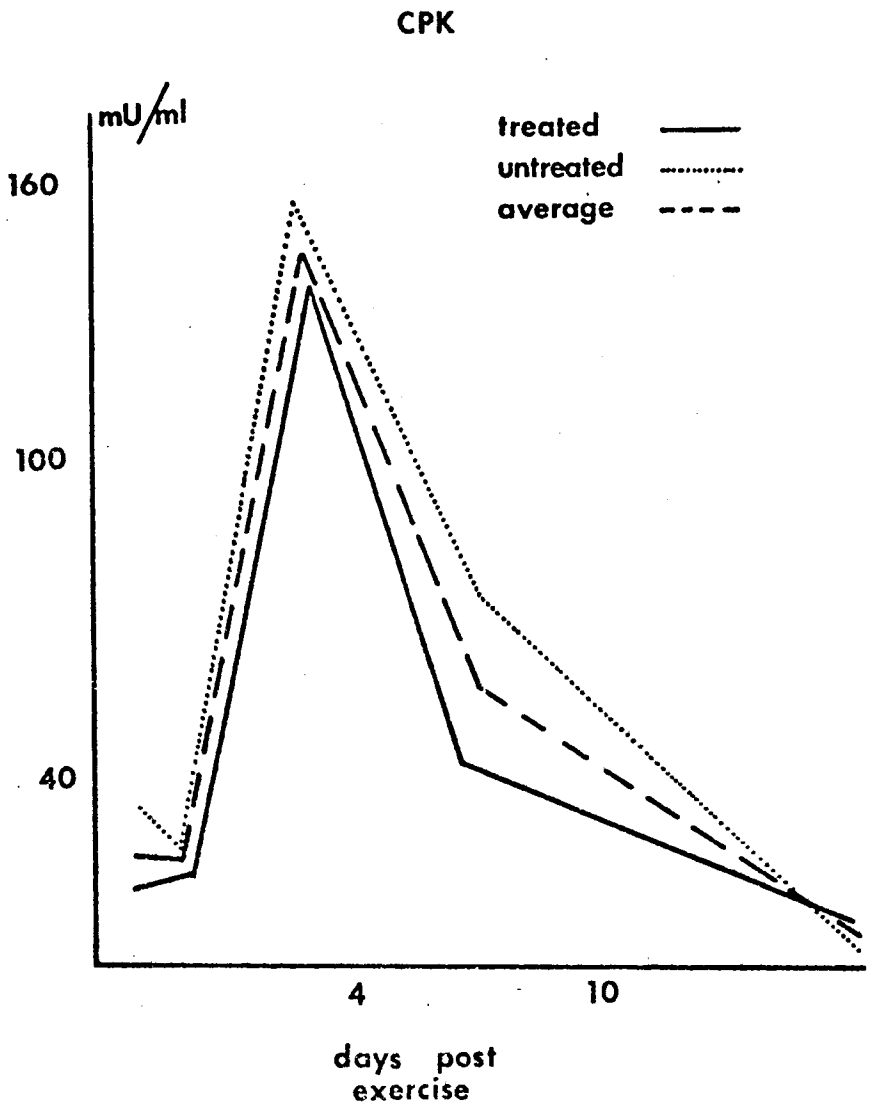


Figure 75: Creatine phosphokinase levels in treated and untreated tsessebe after exercise.

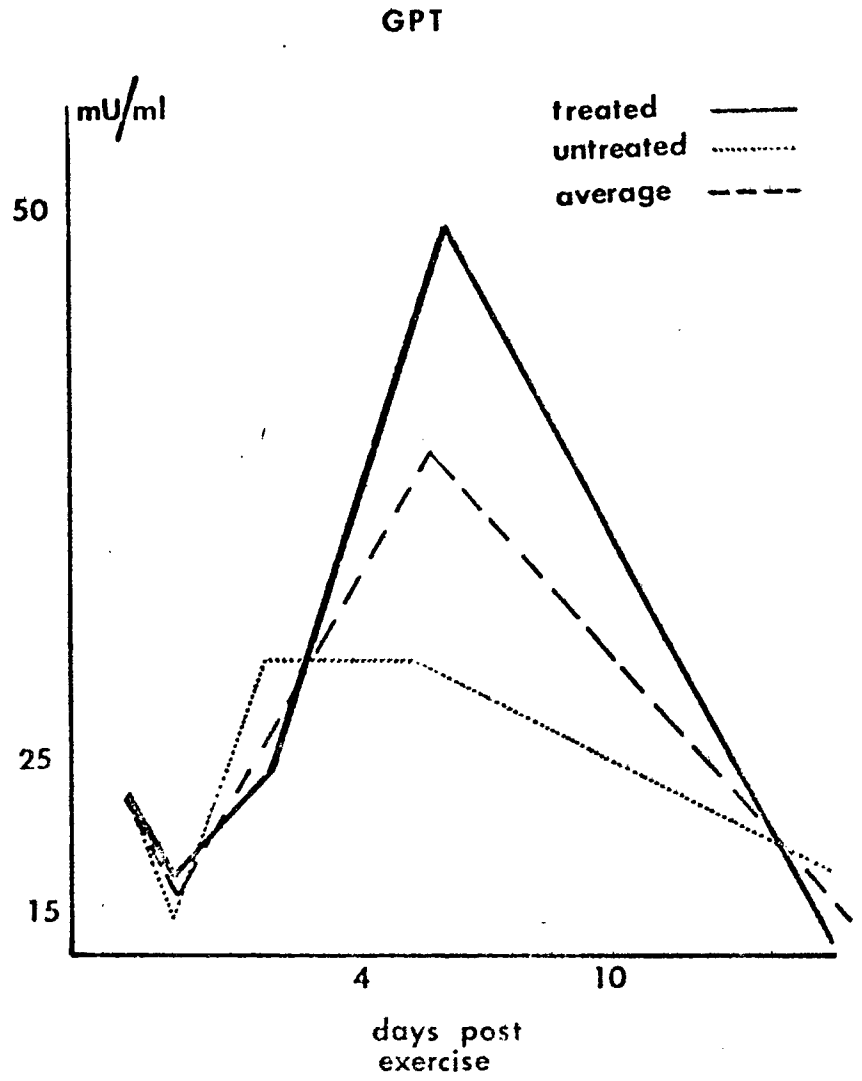


Figure 76: Glutamic pyruvic transaminase levels in treated and untreated tsessebe after exercise.

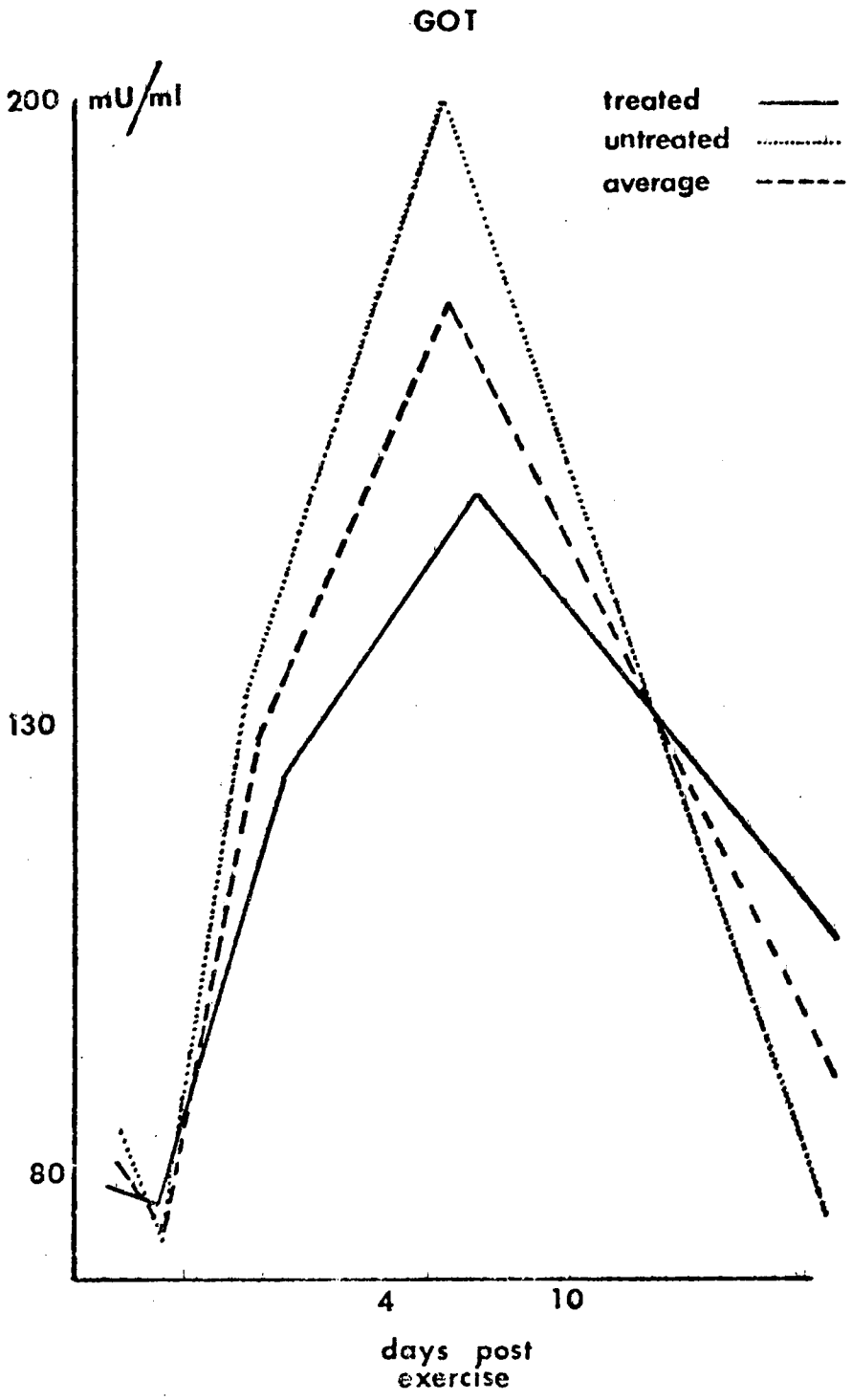


Figure 77: Glutamic oxaloacetic transaminase levels in treated and untreated tsessebe after exercise.

Table 20: Ranges of enzyme levels in tsessebe after exercise.

	$\bar{x}$	low range	high	S_x
after exercise				
GOT treated	79,67	61	106	19,15
untreated	84,00	76	89	5,72
GPT treated	20,67	15	30	6,65
untreated	21,00	17	25	3,27
CPK treated	17,00	16	19	1,41
untreated	37,33	11	68	23,47
LDH treated	458,33	376	529	63,00
untreated	344,67	313	395	35,98
15 minutes post exercise				
GOT treated	76,67	57	112	25,04
untreated	72,67	59	91	13,47
GPT treated	16,67	13	23	4,50
untreated	15,33	11	21	4,19
CPK treated	19,67	11	25	6,18
untreated	36,67	11	54	18,52
LDH treated	425,00	407	445	15,58
untreated	341,00	222	413	84,76
1 day post exercise				
GOT treated	123,00	98	159	26,09
untreated	130,33	45	173	60,34
GPT treated	21,67	19	25	2,49
untreated	28,33	18	42	10,08
CPK treated	139,67	99	186	35,74
untreated	158,67	32	236	90,29
LDH treated	416,33	244	629	159,74
untreated	916,67	620	1 447	375,88
4 days post exercise				
GOT treated	155,67	15	240	100,12
untreated	196,00	122	235	52,35
GPT treated	50,00	29	64	15,12
untreated	28,00	21	35	5,72
CPK treated	42,33	20	68	19,74
untreated	75,67	16	142	51,65
LDH treated	515,33	360	770	181,52
untreated	628,33	360	883	213,73
37 days post exercise				
GOT treated	109,00	68	170	43,98
untreated	75,00	68	85	7,26
GPT treated	13,00	11	15	1,63
untreated	14,33	13	15	0,94
CPK treated	14,00	9	19	4,08
untreated	6,00	2	13	4,97
LDH treated	371,33	357	385	11,44
untreated	392,33	284	470	78,97

level. Recovery of these levels in the untreated group was slower. The ranges show little diversion from the mean values

(Fig. 78).

Differences between treated and untreated groups were compared statistically for each of the above enzymes. The Wilcoxon matched pairs signed rank test was applied and the results were as follows (n = 5 in all cases):

GOT: T(P,N) = 1, T(N,N) = 14

GPT: T(P,N) = 8, T(N,N) = 7

CPK: T(P,N) = 5, T(N,N) = 10

LDH: T(P,N) = 6, T(N,N) = 9

None of these results were statistically significant. All points were taken into consideration, and not only the peak enzyme activity period which usually occurs at seven days after exercise. The sample size was in each case too small to be subjected to meaningful statistical analysis. The graphs indicate GOT and LDH to have the greatest differences between treated and untreated groups at the peak period, with negligible differences in the CPK and GPT values.

ELECTROCARDIOGRAM

ELECTROCARDIOGRAM results show no particular abnormalities relating to any one animal. All animals showed tachycardia with a tendency to extrasystoles. High T-waves indicated a hyperkalaemia which was reflected in red discolouration of the serum indicating the presence of blood and muscle pigments.

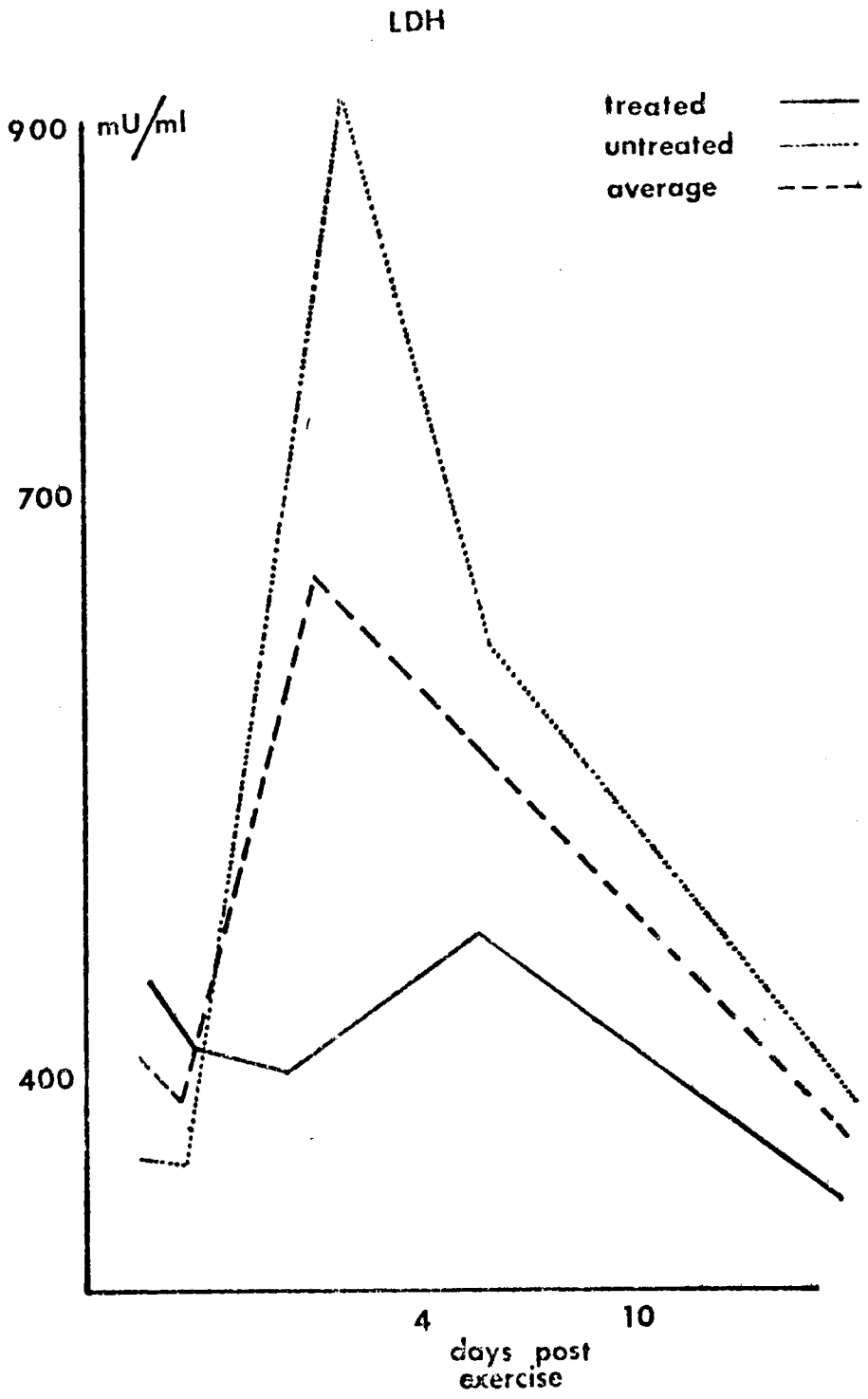


Figure 78: Lactate dehydrogenase levels in treated and untreated tsessebe after exercise.

HAEMATOCRIT

ALL haematocrit values show some decline during the first 15 minutes after capture, i.e. from approximately 50 to 46 percent (Table 21).

Table 21: Haematocrit values in tsessebe after forced exercise.

animal no.→	1†	2††	3††	4††	5†	6†
after capture	-	48	54	53	45	46
15 min. later	-	48	45	50	-	42

† treated

†† untreated

DISCUSSION

DERIVATIVES from the B group of vitamines form co-enzymes which play an important part in the process of biological oxidisation such as the oxidative carboxylation of alpha-keto acids such as pyruvic acid. When there is a deficiency of these co-enzymes, pyruvic and lactic acid accumulate in the body tissues and fluids while they cannot be metabolised.

Deaths of wild animals during the acute stage of capture myopathy have been ascribed primarily to an accumulation of lactic acid and the effect of the resulting low pH on the heart and blood vessels (Chapter Four and Six). On this basis the treatment of animals after capture with those

factors likely to enhance the biological oxidation and breakdown of lactic and pyruvic acids would appear to be a rational therapy.

The exercise on a track as described in Chapter Two (in contrast to capture in the wild) proved to be sub-lethal so that criteria other than survival had to be established to gauge the effect of therapy. Raised values of the various enzymes such as LDH and GPT have been described as occurring as a result of capture stress (Chapter Ten), being generally accepted as reflecting damage to muscle, heart and parenchymatous organs, and the rise in enzymes such as CPK and LDH has been indicated (with potassium) as reflecting increased permeability of the cell membrane (Gericke and Hofmeyr 1976). It seems therefore logical to accept that therapeutic interference that depresses the rise of such stress indicators is at least partially effective in countering the effects of stress and preventing capture myopathy.

It should also be noted in the general context of the track experiments that a line of treatment that shows little effect on the results of a run under the conditions obtaining on the exercise track may yet be effective under the more strenuous and indeed lethal conditions in the wild. For instance, the animals treated in the Kruger National Park, the bicarbonate infusion made 100 percent difference between survival and death under these conditions, while at the lower hydrogen ion concentrations resulting from

a run on the track, both treated and untreated controls survived.

HYDROCORTISONE

Introduction

THE object of the tests was to examine the effect of the intravenous administration of a large dose of hydrocortisone on exercise-induced stress. Hydrocortisone is not only recognised as an anti-stress remedy, but also as an agent effective in the relaxation of vasospasm. Synthetic glucocorticoids have been established as effective in counteracting capture stress in sheep (Gericke and Hofmeyr 1976).

Materials And Methods

EIGHT eland were available and were divided into two groups. The two groups acted alternately as controls. The one group was treated on the first run, and the other on the second run carried out about one month later. The method of chasing and sampling is described in Chapter Two.

Results And Discussion

HYDROCORTISONE therapy in eland was inconclusive. A comparison of the results of the first run was as follows:

Figure 79 shows that the levels of enzymes in treated and untreated animals was virtually the same with the exception of GPT. Should these results and those obtained with B-complex vitamins be reproducable then the apparent effectiveness with regard to GPT may be valuable. The B-complex appeard to be at least effective in respect of GPT so that the possibility may exist that treatment with the B-complex and hydrocortisone may complement each other.

The results of enzyme estimations when a second run was made a month after the first, were strikingly different. Statistically the results are the following:

GOT: $t_3 = -8,1026^{***}$

GPT: $t_3 = -3,149^{*}$ (nearer to **)

CPK: $t_3 = -14,101^{***}$

LDH: $t_3 = -1,812$ not significant at 10 percent

The negative value indicates that the second (treated) peak was lower than the first (untreated) peak.

The results are further depicted in Fig. 80. Both statistically and as read from the graphs these results appear to be highly significant. As such they are perhaps an excellent illustration of how misleading results can be, especially in the context of treating wild animals when the numbers available are usually few, and the conditions seldom amenable to proper control tests, i.e. no group of eland was run twice without being treated at any time.

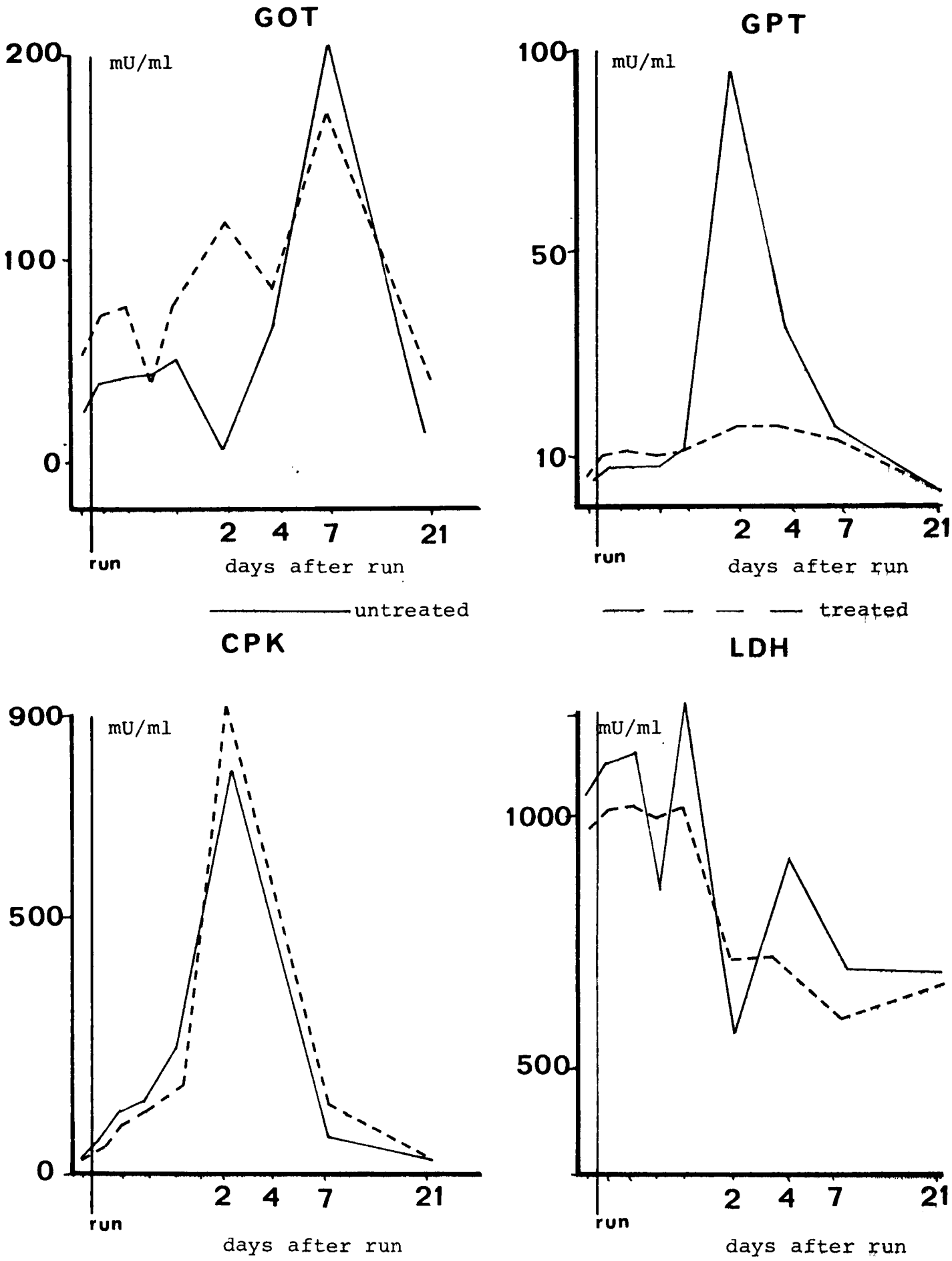


Figure 79: Enzymes in treated and untreated eland after first exercise.

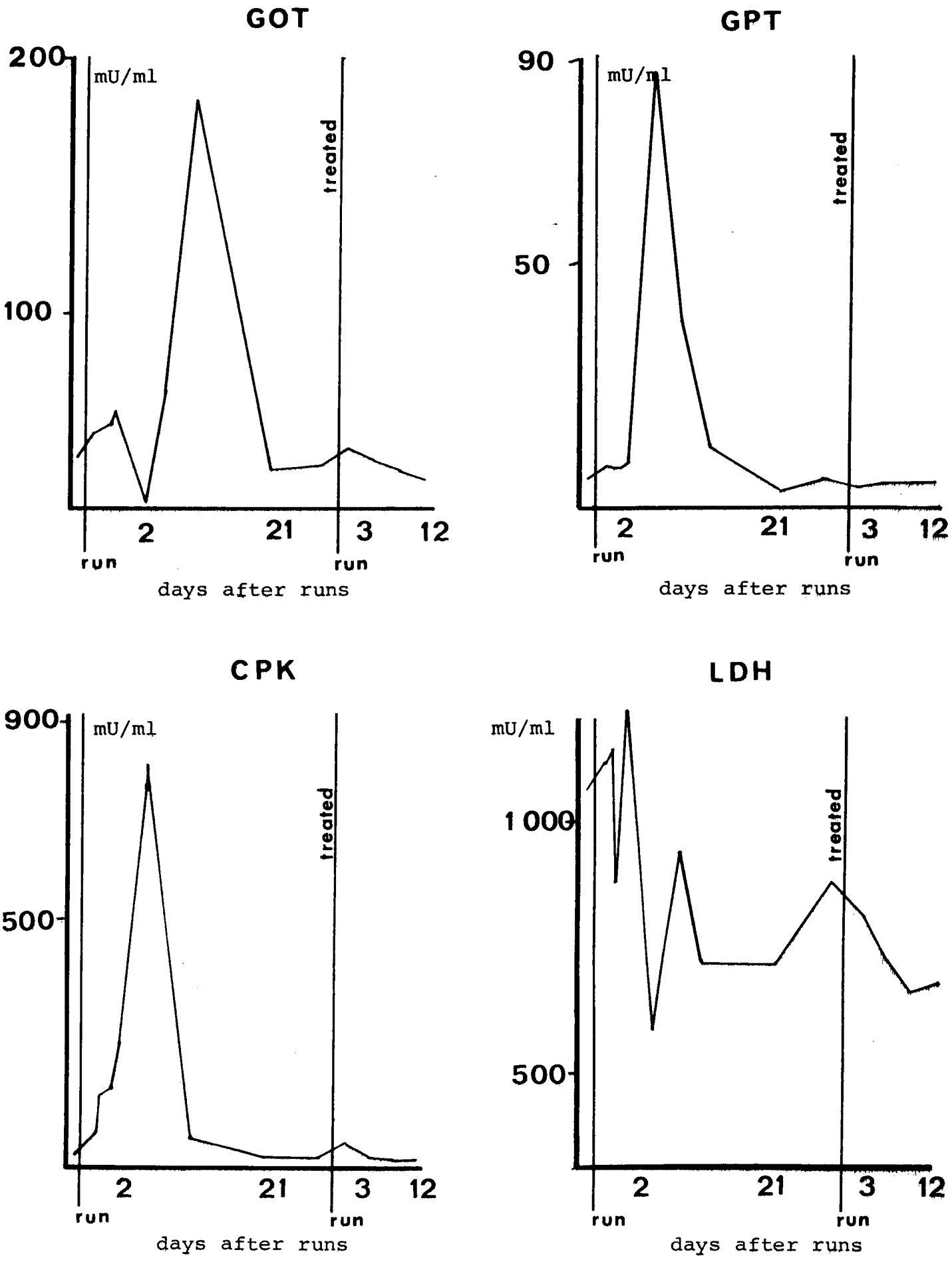


Figure 80: Enzymes in eland showing difference between first and second (treated) exercise.

If we look at the results of the second group which was run on the same day, that is the group which was treated at the time of the first run (broken line in Fig. 79) and not treated after the second run a month later (Fig. 81) the results are seen to be not markedly different from those of the first group which was originally untreated, and treated at the time of the second run. Statistically the results were as follows:

GOT: $t_3 = -4,1687^{**}$

GPT: $t_3 = -2,505^{*}$

CPK: $t_3 = -9,558^{***}$

LDH: $t_3 = -4,218^{**}$

The negative value indicates that the second (untreated) peak was lower than the first (treated) peak.

Clearly there is a factor involved here that is considerably more effective than that of hydrocortisone therapy (see below).

PREVENTATIVE MEASURES

INTRODUCTION

THE principle of training as a means of adapting muscles and the general constitution to locomotory stress is generally accepted. The different levels of lactate, pyruvate and hydrogen ion concentration in the blood of horses at different levels of training was established by Engelhardt, Hörnicke, Ehrlein and Schmidt (1973). Lactate and pyruvate

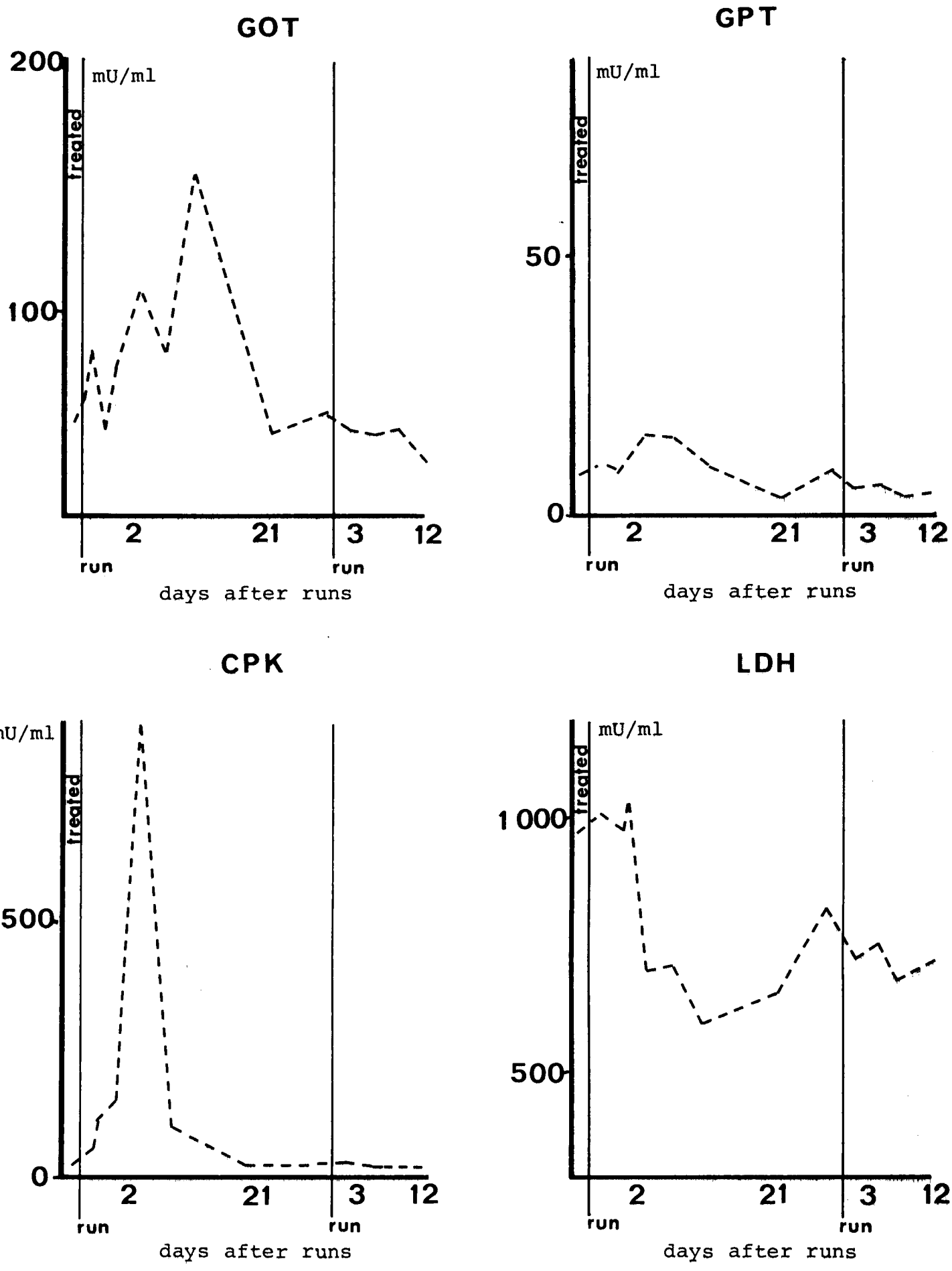


Figure 81: Enzymes in eland showing difference between first (treated) and second exercise.

concentrations and ATP, etc. have been determined in relation to age and training in standardbred horses by Lindholm and Piehl (1974), and the physiological and biochemical response of standardbred horses to exercise has been investigated by Lindholm and Saltin (1974) who established that glycogen utilisation was most intensive during the first few work bouts. Holloszy (1967) has established that training doubled the capacity of the mitrochondria to oxidise pyruvate. Changes in activity of muscle-specific enzymes (such as CPK) was related to training in thoroughbred horses by Riethmüller and Wels (1972).

Materials And Methods

Four black wildebeest bulls kept in one of a series of 25 ha paddocks were driven with the use of one truck and a dozen helpers on foot (Chapter Two).

The capture corral was constructed of varying lengths of plastic polypropylene sheeting 1,5 m high using poles and wire for support. The animals were driven through part of the structure which was erected in the paddock early in each month. Samples were taken only at the final run each month when actual capture took place. Peripheral blood samples only were taken, the original object of the monthly capture being blood analysis for mineral content.

Results

THE first capture, performed without preliminary training, resulted in the death of two out of the four wildebeest, one at least partly from injury. No further deaths occurred during the remaining six months of the experiment.

The blood samples collected from the four animals at the first capture in May showed darkly discoloured plasma. The plasma samples on the subsequent run showed only sporadic light discolouration, after which no frank discolouration of the plasma occurred.

Discussion

LOCOMOTORY stress, induced without preliminary training, results in high blood enzyme levels which take several weeks to decline, the release of blood and muscle pigments into the plasma, and often considerable mortality.

The eland experiment, and to a lesser extent those carried out on zebra, showed that enzyme levels during a later or subsequent chase are mostly only a fraction of the original values. Eland could not be run on a monthly basis for test purposes as enzyme levels soon became negligible.

Discolouration of the blood plasma with blood and muscle pigments has been shown to develop progressively in relation to the distance covered (see experiments on blesbok).

They were also found in captive sable after a short period of intensive muscular strain. The release of myoglobin has been shown to occur from exercise which is unusual rather than excessive, such as after only fifty squat jumps in marine recruits, who were able to perform many times this number of squat jumps during the subsequent training period (Chapter Nine). Similarly red discolouration of the plasma in the wildebeest was seen mainly after the first run, and not in later runs at monthly intervals with gentle semi-monthly runs in between.

The principle whereby animals are exercised, and familiarised with the capture structure affects the capture procedure in three principal ways.

(a) Training resulted in reduction of potentially lethal glycogen stores, and reduced liberation of blood and muscle pigments which cause nephrosis and death from renal failure, and high potassium levels with deleterious cardiac effects.

(b) Taming - the familiarisation by the animals to the wings (and later also the neck) of the corral system appeared to reduce fear and therefore adrenaline secretion. Sympathetic discharge increases glycolysis, induces vasospasm and excessive flight reaction, so increasing the danger of shock and trauma.

(c) Tempo - there is a reduced speed of chasing after acclimatisation of the animals to the capture corral. They are accustomed to running through the wings and neck which they undertake more readily while they are able to see

open veld and bush on the far side, and the structure is in their vicinity for several weeks so that they may in fact graze in the wings. It is related (McDonald†) how an entire day spent in fruitless efforts to drive a herd of blue wildebeest into a corral, ended in the animals entering of their own accord to seek shelter from cold winds during the night.

This method involving the prior training of animals before capture appears clearly applicable to the Transvaal provincial reserves where the animals are kept in restricted surroundings. The tests reported here indicate that the use of this method should result in considerable saving of animal lives from accidents, capture myopathy, and probably from sympathetic hypertonus after capture. It enables the improved techniques using corrals constructed from hessian or polypropylene fibre to be used instead of nets, and enables the animals to be rested in corrals immediately on capture instead of being handled in nets and subjected to stresses of close confinement. Lastly the method of driving into corrals enables family units to be captured and moved with reduction of fighting, stress, and more ready adaptation to new surroundings after relocation.

† R. McDonald, formerley Professional Officer at S.A. Lombard Nature Reserve.

CHAPTER SIXTEEN

GENERAL DISCUSSION

CAPTURE MYOPATHY AND SHOCK

CAPTURE myopathy and shock have certain features in common, even during the early stages of the former. Furthermore, untreated or lethal capture stress will inevitably manifest all the clinical signs of shock during the final phases prior to death. Since shock may arise from a number of causes unrelated to capture stress, it is important to be able to differentiate between the two, particularly during the early phases, when treatment is likely to be effective.

Shock is usually described as a syndrome including cardiovascular disturbances that, unless treated in the early stages, rapidly becomes irreversible, leading to peripheral circulatory failure, stagnant and anoxaemic anoxia, lack of venous return, central anoxia, coma, respiratory failure and death. It may be induced in a large number of ways, such as by repeated small haemorrhages, extensive bruising of tissues, tourniquets, cooling of the abdominal cavity, and reduction of the blood pressure by histamine or by other means. Shock is associated with persistent hypotension, while hypotension maintained for a period of two to three hours will produce shock that will result in death if left untreated.

In considering the effects of exercise or capture, a number of subsidiary factors should be considered that are likely to contribute to the stress condition. Among these are psychological stresses due to anxiety or fear, abnormal posture during restraint and the effects of tying the legs, overheating and bloating due to restraint, and the effects of any drugs that may be used. These subsidiary factors in themselves will affect the cardiovascular, respiratory, and thermoregulatory mechanisms and thus contribute to the state of stress or damage, especially when the thermoregulatory mechanisms are disrupted by drugs, autonomic responses are exhausted by fear, transudation of blood results from trauma, bloat from abnormal posture affects respiration, and physical rupture of muscle fibres has occurred.

Where care is taken to ensure that the impact of these subsidiary factors is kept to the minimum, the chances of recovery from the principal stresses due to actual capture are improved.

Causes

UNDER capture conditions, shock may eventuate from a number of causes such as: Idiosyncratic reaction to injected compounds or anaphylaxis; overdosage of capture drugs leading to cardiovascular derangements; massive adrenaline output; extensive bruising or rupture of muscle fibres; anoxaemia due to hypoventilation; hyperthermia; nervous exhaustion, also more general causes such as adrenal exhaustion and endo-

toxins from bacterial multiplication. Shock in wild animals may be caused by haemorrhage, especially haemorrhage into the tissues from trauma, from the toxins of infections, from incorrect dosage with capture drugs, and from large doses of analeptics or adrenaline, from bone fractures or dystokia, or from intestinal obstruction or malfunction.

Differential Diagnosis

Diagnosis of disease in the wild animal is notoriously difficult. Not only are the symptoms associated with specific conditions seldom exhibited, but the animals cannot be approached for reading of pulse pressure or rate, or for temperature measurements, and for blood or urine samples which cannot be taken without risk of precipitating the disease condition, or the danger of causing death from trauma.

The general clinical signs of shock, insofar as these may be ascertained in the captive wild animal, are cyanosis, coolness of the extremities and narrowed pulse pressure. There is also oliguria or anuria.

Principal Types Of Shock

The physiological mechanisms of principal types of shock, derived mainly from experimental work on dogs, have been summarised by Lillehei, Longerbeam, Bloch and Manax (1964), whose classification of the different forms of shock is followed here. These authors point out that irreversible

shock results from irreversible ischaemia or anoxic changes in one or more organ systems of the body.

1. Cardiogenic Shock

This is the type of shock described principally in man as a result of myocardial infarction. This can be duplicated experimentally in dogs with similar resultant clinical symptoms. Cardiac output is decreased, and there is a steep increase in the total peripheral resistance, metabolic acidosis and a decreased venous return (Richardson 1963). The acidosis and increased peripheral resistance are, however, secondary to the cardiac catastrophe. In acute capture stress there is reduction in cardiac output, an increase in the resistance of the pulmonary circulation, and a fall in systemic pressure. These are not due primarily to cardiac failure, and the various parameters return to normal levels when the acidaemia is rectified. The cardiac dysfunction is due to the acidaemia in this case, although the acidaemia is inevitably exacerbated by the reduced cardiac output.

It is clear therefore, that although the symptoms are similar, and if left untreated the course of events in capture stress will be the same as that of cardiogenic shock, cardiac failure is rarely the primary cause. As a result, the condition of capture stress may be rectified if the pH is artificially restored so that the normal heart action is re-established, and the increase in vascular resistance reduced to normal levels. This results in an increased venous return, normalisation of tissue perfusion, and relief of peripheral

vascular disturbance.

2. HAEMORRHAGIC SHOCK

THIS is induced by bleeding dogs for a few minutes to reach a blood pressure level of 35 mm Hg, with small amounts of blood collected over the subsequent hour.

Symptoms are hyperkalaemia and acidaemia. The blood pressure returns to near normal levels after infusion, but this level is not maintained. The cardiac output is lowered due firstly to the increase in peripheral resistance, but then also to the fall in blood pH. The blood flow to the kidney and liver has been shown to be decreased to a greater extent than the blood flow to other organs, the heart and brain receiving a larger share of the reduced circulation (Lillehei, Longerbeam, Bloch and Manax 1963). During this stage the haematocrit rises and the blood volume is decreased due to loss of plasma from the hypo-circulation.

Later on, whole blood is also lost. This occurs as a haemorrhagic suffusion of the mucosa of the bowel which leads to necrosis, also of the liver and kidneys. The red cells return to the circulation through denuded areas of the intestinal tract to appear as plasma-haemoglobin (Lillehei 1958).

Thepresence of pigments is seenin stressed animals. In these, however, the discolouration of the plasma is at least partly due to myoglobin. The systemic blood pressure is

only slightly depressed, and the acidosis tends to induce an intense tachycardia. Blood volume determinations show a reduced circulating volume. The haematocrit shows variations rather than a rise, as it tends to be raised at the onset, drops during the subsequent hours, and then rises after infusion (Chapter Eight), the results of infusion in capture stress are likely to be permanent with a restoration of normal functions. In haemorrhagic shock, the improvement after infusion is temporary.

3. Endotoxin Shock

This condition is due to the endotoxins found in the cell walls of gram-negative bacteria which have a sympathetic action causing intense vasospasm in arteries and veins. This type of shock resembles certain manifestations of capture stress more closely in that no blood has been abstracted from the circulation, and as the main cause of the shock is vasospasm. The injection of the endotoxin, a complex glycopolysaccharide causes blood pressure to fall transiently as a result of hepatic venous constriction in turn due to histamine release (MacLean and Weil 1956).

Here also there is vasoconstriction in various parts of the body, particularly in the splanchnic area, followed by a progressive dilatation of the arterioles and venules and congestion of the capillary beds (Zweifach 1961). Eventually there is tissue degeneration and necrosis due to stagnant anoxia.

The symptoms are progressively increasing plasma haemoglobin. This contrasts with animals in capture stress where the red discolouration of the plasma tends to fall as the small molecules of the myoglobin are excreted, depending on the degree of stress and possible oliguria. The hypotension is caused primarily by the loss of plasma fluid into the tissues, rather than from a primary reduced cardiac output due to low blood pH and pulmonary vasoconstriction.

4. Adrenaline Shock

It has long been established that prolonged infusion of adrenaline can by itself cause fatal shock in the dog. The amount infused is large, and in the order of 17 µg per kg per minute over 90 minutes (Lillehei and MacLean 1959). This technique produces an intense ischaemic anoxia which is eventually followed by congestion and stagnation. The pattern of symptoms is similar to those of the other types of shock described, and also the autopsy findings with severe congestive changes in kidneys, liver and intestines.

Our infusions of adrenaline did not lead to shock but were of a very much smaller order, namely 20 gamma per kg per hour. This quantity of adrenaline is certain to be far less than that secreted by the animal under conditions of stress, and it did not result in a marked rise in the systemic blood pressure, but did apparently affect the kidneys. The infusion of adrenaline into dogs caused a fall in blood pH, and a rise in blood potassium, also evident in our infused animals. The correction of the blood pH and the electrolyte

shifts and the eventual blood volume deficits made no difference to the fatal course of the experiments in dogs. One of our animals was lost subsequent to adrenaline infusion and when the potassium rose to a particularly high level. The kidney showed congestion with tubular accretions of pigments.

Progressive Stages In Capture Myopathy

ANIMALS that succumb to capture myopathy eventually die of shock. When the blood pressure falls to shock levels, ie. about 70 systolic, then death from shock will occur irrespective of the origin of the hypotension. Soon, about two to three hours after the commencement of hypotension, there will be a progressive reduction of venous return due to capillary damage and loss of vasomotor control. The haematocrit rises due to the escape of plasma from the blood, again due to increase in the capillary permeability and consequent loss of serum albumin. Increased blood viscosity increases circulation time, thereby decreasing the oxygen content, thus causing further capillary damage and further loss of blood pressure. Eventually the oxygen carried by the blood falls to levels that are incompatible with central nervous function, and the vital functions such as respiration fail.

VASOSPASM

THREE findings indicate the presence of vasospasm in captured animals. Firstly the rise in blood urea nitrogen, urea and creatinine, together with deaths of animals from apparent renal failure, secondly, the rise in pulmonary arterial pressure, and thirdly, the low pH and PO_2 and the high PCO_2 in capillary blood.

The renal vasoconstriction may be duplicated by exogenously administered catecholamines and presumably is equally prone to occur from endogenously produced products of sympathetic hypertonus. Infusion of adrenaline did not, in the quantity administered, produce marked change in the pulmonary arterial pressure (Chapter Six), although the pulmonary vessels are believed to fall under the control of the sympathetic nervous system (Fishman 1964). The rates of infusion used were sufficient to induce apparent renal vasospasm manifested by a rise in the blood levels of urea, blood urea nitrogen and creatinine.

Animals suffering from capture stress enter into states of shock with symptoms predominantly of pulmonary oedema (acute) or renal failure (delayed) without marked prodromal symptoms of low blood pressure. These results confirm the experience in clinical human medicine that blood flow rather than blood pressure is the key to the maintenance of circulatory integrity (Shoemaker and Brown 1971) although historically, hypotension has been considered the major clinical sign of the advent of

an irreversible circulatory state leading inevitably to shock, coma and death. Therapies that have relied on vasopressor agents for maintaining the blood pressure have had little or negative success, as blood pressure maintained in this way is done at the expense of blood flow to vital parenchymatous organs and the alimentary tract. When this is done, the subject will suffer a precipitate and irreversible fall in the blood pressure when eventually the anoxia and an accumulation of tissue metabolites destroy the integrity of the closed blood vessels, or eventually die of renal failure, due to degenerative changes in the renal tubular apparatus. It has been pointed out the vasoconstriction does not occur uniformly throughout the vascular tree, and that rather unequal constriction produces maldistribution of both blood volume and blood flow among organs as well as within organs (Shoemaker 1967).

The constriction of parts of the vascular system under the effect of sympathetic nervous impulses is well-known and is responsible for familiar phenomena such as the rise in blood pressure and blanching of the skin on injection of adrenaline and noradrenaline. Similar results may be obtained by chemoreceptor stimulation (Kahler, Goldblatt and Braunwald 1962, Rudolph and Auld 1960). Venospasm may also be induced by injection of histamine (Hinshaw, Vick, Jordan and Wittners 1962, Graham and Lewis 1953).

Among the vessels that constrict under the influence of sympathetic nerves are the pulmonary veins (Eliakim and

Aviado 1961). This constriction of pulmonary veins under strong continuous adrenergic tonus, is likely to lead to pulmonary oedema. The pulmonary vasoconstriction in particular is mediated by alpha receptors, at least under certain experimental conditions (Stern and Braun 1966). The fact that we were able to alleviate such vasoconstriction of the pulmonary vessels by restoring a near normal blood pH condition (Chapter Six) suggests that the constriction is likely to have been mediated in the first place through chemo-receptors and in the second by sympathetic nervous reflexes. A direct effect of the low pH, especially in pulmonary arterial constriction cannot be excluded.

At one time hypotension and shock were considered virtually synonymous. Hypotension inevitably led to shock, while death from shock was preceeded by hypotension. However, it is more often the cause of the shock that is responsible for hypotension, such as trauma with interstitial fluid loss, cardiac failure, or loss of blood. Later it was realised that shock may exist together with a normal or even an elevated blood pressure. Conversely, a low blood pressure may exist in cases where there is no shock. The latter usually exhibit a wider pulse pressure and warm, dry skin and extremities (Page 1961).

Shock is engendered by an insufficiency of blood flow leading to irreparable dysfunction in certain areas or organs, rather than a low blood pressure. These areas can be classed key vascular beds that are primarily involved in the vasoconstr-

ictor response (Nickerson and Gourzis 1962).

The continued blood flow in these vital areas is the major determinant of survival in shock, and the maintenance of the blood pressure may, in fact, be deleterious if it is achieved by vasoconstriction and further reduction of blood flow through essential organs. Vasoconstriction in organs such as the lung and kidney occurs in stressed animals which exhibited little or no change in the systemic blood pressure. Thus the rise of blood pressure of the pulmonary artery leads to death from pulmonary oedema in untreated zebra, and the infusion of adrenaline in sable antelope caused reduced renal function and death apparently from renal failure.

The secretion of large amounts of sympathomimetic amines under condition of intensive exercise and fear is generally accepted as is the fact that these substances can cause changes in the calibre of blood vessels. The action on the blood vessels is direct and no intermediary intact nerve supply is required. Other factors appear to play a part. Experiments on dogs where the blood flow in the hind limb was measured during haemorrhage and shock indicated that an initial reduced hind limb vascular conductance occurring during haemorrhage could be ascribed to stimulation of alpha receptors, but a later reduced conductance after reinfusion of the abstracted blood could not be so ascribed and was therefore thought to be due to other factors (Halmagyi, Goodman and Neering 1969).

Shock may be readily induced by the administration of both histamine and endotoxin, the first only resulting in an immediate and precipitate fall in blood pressure. Haemodynamic changes that occur in endotoxin shock are a rise in the portal vein pressure, a pooling of blood, a reduction in cardiac output and a decline in arterial systemic blood pressure (Halmagyi, Starzecki and Horner 1965). The rise in the portal pressure (and therefore the reduced venous return to the heart and pooling of blood) caused by histamine could be prevented by alpha-blockade of the histamine constrictor action on the hepatic venous system (Brake, Emerson, Wittmers and Hinshaw 1964).

A similar action obtains against the histamine released by endotoxin at the site of the hepatic vein, although the effect of this histamine on the peripheral circulation appears to be counteracted by the action of constrictor agents also released by the endotoxin (Lillehei and MacLean 1959).

Reduction of vasoconstriction as a factor for survival, if carried out shortly after the loss of blood, was demonstrated during experiments on dogs. A significant fact was that the blockade of alpha receptors alone had no survival value under the circumstances of the experiments. The dilated blood vessels have a large capacitance so that to constitute an effective factor for survival, the alpha blockade must be accompanied by fluid replacement (Lotz, Beck and Stevenson 1955).

Renal vasoconstriction in kidneys removed from healthy donor dogs was also established. This vasoconstriction rendered the kidneys useless for auto-transplantation. The constriction was ascribed to a short period of warm ischaemia while the renal vessels were clamped prior to removal. When excision occurred without clamping of the renal vessels the kidneys were capable of life-sustaining function (Frost, Ackermann, Tyree Finch and Manlove 1970). These trials suggest that an ischaemia may in itself induce vasoconstriction in the renal vessels which then prevents perfusion and in turn results in tissue necrosis.

The state of the blood vessels in skeletal muscle under stress conditions is uncertain. The arterial conductance in the hind limb of dogs remained unchanged during haemorrhage (Halmagyi *et al* 1969), while intra-arterial alpha blockade of the forearm after noradrenaline infusion induced an increase in flow of 90 percent in the treated limb, an increase comparable to that resulting in blockade of the deep nerve of the forearm (Allwood and Ginsburg 1961). This increase in conductance was ascribed to the blockage of sympathetic vasoconstrictor responses and not to vasodilator responses; the response of the vessels to the limb having both dilator and constrictor components, although the response to noradrenaline is purely vasoconstrictor. Vasoconstriction has been shown to occur as a result of a fall in systemic blood pressure (Bowman 1959).

The degree of vasoconstriction in the limbs of our stressed

animals is not known, or if such constriction was general or local. The local increase in acidity determined by pH probe tests in the legs of zebra after forced exercise, indicates local increases in acidity which have been ascribed to accumulations of lactic acid.

Such increases may be due to a decreased perfusion of that part of the limb as a result of vasoconstriction or through a lowered perfusion pressure. The tests were made at least 15 minutes after the termination of forced exercise and could therefore not readily be related to a continuing build up of lactic acid. Residual muscle tension may, however, have played a role either in continuing production of lactate or in preventing a normal blood flow through certain areas of muscle tissue. It is tempting to assume that such local increases in lactic acid concentration occur during the actual chase with resultant mineralisation of muscle fibres. Also that such a loss of normal muscle fibre activity (and probably the ability to relax) on a localised basis, is the cause of the rupture of muscle fibres which has been observed on the *post mortem* examination in animals that have died after locomotory stress.

ADRENALINE EFFECTS

INTRODUCTION

UNDER normal circumstances adrenaline exerts a positive

inotropic effect on cardiac muscle, and dilates blood vessels in those parts of the body associated with emergency action. It is generally accepted that under stress conditions when the internal environment of the body is radically altered, these effects of adrenaline may be prevented or may be deleterious. These conditions are broadly:

(a) When the normal effect of adrenaline is mediated through factors that cannot operate under the prevailing conditions;

(b) when the effects of adrenaline are excessive and therefore counter-productive, and

(c) when the effects of adrenaline are unduly prolonged due to unnatural circumstances and become harmful.

These groupings will be discussed below. In this discussion the term adrenaline will be used in a broad sense including sympathetic effects in general, whether mediated through the adrenal medulla or through discharge of the sympathetic nerve endings.

Cardiac Musculature

The effect of adrenaline on the cardiac musculature, particularly that of the ventricular muscle fibres, is believed to be mediated by cyclic AMP (Tsien and Weingart 1974). While it is unlikely that this represents all aspects of adrenaline effect, it does indicate that the mechanism is associated with the highly complicated energy cycles that

are involved in muscle contraction and energy flows, which are regarded as highly susceptible to changes in the homeostasis of the internal environment.

The heart rate, under the conditions of acute stress and low pH, is excessively high. The rate is clearly too high to permit adequate diastolic filling. At the same time, and perhaps paradoxically, cardiac dilatation does occur especially in zebra. This has been determined by auscultation and established at autopsy.

The classic experiments on the perfused heart indicates that the heart stops suddenly when the pH falls below levels approximately 6,4 and is already impaired at a pH below 7,0. It seems probable that the inotropic effect of adrenaline on cardiac musculature fails when the pH of the blood is reduced for reasons similar to the failure of normal mechanisms in relation to skeletal muscle at low pH, for the reasons already discussed. These are the abrogation of normal enzyme mechanisms by either the direct effect of pH change on enzymal or other mechanisms or the indirect effect mediated by the immobilisation of calcium ions. The effect of excessively high potassium (normal about 3,5 to 5 m-equiv./l) causes cardiac abnormalities which are, in fact, similar to those of low calcium (mainly a prolonged QT-interval in the electrocardiogram tracing - Chapter Five). It is possible that the high blood potassium, under conditions of intravascular haemolysis demonstrated as occurring in our experimental animals, has effects that are

additive to the calcium effects. Under these conditions it is unlikely that adrenaline can have any salutory effect on cardiac function or that the inotropic adrenaline effect remains operative.

Vasospasm

The vasoconstriction induced by adrenaline is valuable under acute conditions to render the circulatory blood available to the skeletal musculature and the brain. These conditions obtain under emergency circumstances. The operative mechanisms are well known, and are mainly concerned with the reduction of blood flow to the vegetative organs of the body, the splanchnic region, the kidney, with concommitant vasodilation in skeletal muscle areas. The sympathetic mechanisms appear to be highly developed in wild animals which are attuned to prodigious and maximum short-term efforts to escape predators. This is virtually an all or none response when the body is all but sacrificed in an effort to escape. The sympathetic discharge clearly has survival under these circumstances. Escape from predation is a short-term affair. The lion or leopard either makes a successful foray or its prey escapes. There is little survival value in graduating the prey response; the animal is prepared to put everything into the physiological scales to escape Hunting dogs and hyaena operate in a different way, although it appears that in any case the hunted animal usually succumbs and is killed. A highly interesting observation has been made (Jack Hopcraft, Athi River). On a

farm in East Africa, when Thompson gazelle were seen to escape from a chase by cheetah, a gazelle - presumed the one chased - was usually found dead on the following day.

Deleterious Effects

Under the extended unnatural circumstances of conventional animal capture deleterious results occur. Even in the relatively short chases by helicopter for remote injections, the effects of adrenaline are prolonged well beyond normal circumstances and in this context excessive. These deleterious effects may be summarised as follows:-

(a) Intensive vasoconstriction of regions of the body resulting in accumulation of metabolites and vascular damage.

(b) Reduced blood flow to parenchymatous organs such as the liver, abrogating important liver action. Reduction in perfusion pressure to organs such as liver and kidney resulting in cellular necrosis.

(c) Excessive glycolysis under relatively anaerobic conditions causing accumulation of lactate-pyruvate and a fall in pH.

Sequestration

Our own experiments including haematocrit studies, PO_2 and PCO_2 measurements before and after infusion and blood volume estimations indicate that large sections of the body are sequestrated from the normal circulation. (This is both a direct adrenaline vasoconstriction effect, and an indirect

one resulting from a reduction in cardiac output through the action of low pH and high potassium on the heart, and therefore an action comparable to that of reduced blood volume and ultimately a rise in the haematocrit in haemorhhage.) As demonstrated in Chapter Fifteen and Chapter Four, the result of restoration of the blood pH by infusion results in lowering of the central blood carbon dioxide, raising of the haemtocrit and increase in blood volume greater than that of the fluid added. These paradoxical effects are the result of the re-establishment of a previously curtailed circulation and therefore may be regarded as the reversal of adrenaline effects. The sequestration of parts of the circulation, particularly on a relatively long term basis in captured animals appears to be a factor in causing ultimately irreversible damage to blood vessels, muscle cells, parenchymatous organs and the kidney.

Damage To Blood Vessels

THE damage to blood vessels results progressively in increased capillary permeability, loss of circulating fluid, reduced circulatory volume, vasodilation with lowering of blood pressure, regional anoxia, central anoxia, loss of vasomotor control, coma and death. This is likely to be the sequence of mechanisms leading to death in animals that die one to 24 hours after capture.

Damage To Muscle Cells

Damage to muscle cells in the stressed animals has been shown to result in the following: Muscle spasm, torticollis, lameness or 'tetraplegia', prostration, liberation of myoglobin into the circulation, rupture of muscle fibres, haematoma formation, pain, swelling, loss of circulating fluid, death from indirect causes associated with recumbency and/or renal failure.

Damage To Kidneys

Kidney damage occurs from a combination of causes involving a reduction in blood supply and accumulation of solid material in the tubules. The lowered perfusion pressure in itself may cause tubular necrosis. A lowered perfusion pressure also appears to facilitate the blockage of kidney tubules with haemoglobin and myoglobin. It may be assumed that the high death rate in animals with symptoms of tubular damage may be ascribed to the fact that quantities of these products which could be eliminated by the healthy body, cause renal failure when the kidney is not fully functional due to: Damage to kidney tubules from greatly reduced blood supply; sub-optimal function due to reduced blood supply; specific action on the kidney through vasoconstriction or the effects of metabolites, and vasoconstriction due to the effects of adrenaline (see experiments with sable antelope), and precipitation of blood and muscle pigments by acid conditions.

The kidneys of stressed animals show a combination of tubular necrosis, tubular degeneration, and blockage by casts of iron-containing pigment. The renal failure complex undoubtedly plays a part in deaths that occur during the sub-acute phase of capture myopathy; which may last for several weeks.

Anxiety And Chronic Adrenaline Effects

WILD animals die in captivity when the conditions of capture have apparently been optimal, i.e. capture stress has been kept to a very low level. Deaths appear to be due to an anxiety syndrome or from so-called nervous exhaustion or nervous stress. Various causative factors have been postulated to account for these phenomena including the effects of increased muscle tension, hyperventilation and adrenal exhaustion.

A useful comparison may be made of this condition with the so-called anxiety syndrome or anxiety neurosis in man, which has recently been recognised to affect clinically some 10 million Americans and is recognised as the underlying cause of the symptoms or complaints of between 10 to 30 percent of the patients of most general practitioners. The symptoms of this condition are frightening to the patient, are associated with intense fatigue andpalpitations, but until recently laboratory tests indicated no specific lesions or conditions (Pitts 1969).

The general symptoms amongst those reported may be compared

to the anxiety condition in various species of animals, and are:

(a) Symptoms in man and in animals - tachycardia, hyperphrenia, trembling, sweating, hyperkinesis, urinary frequency, anoxaemia, diarrhoea, vomiting.

(b) Symptoms in man - subjective symptoms in man are increased reaction to noise, light and heat. The extent to which these affect captive wild animals must remain speculative. Others include fatigue, vertigo, faintness and weakness, apprehension, headache, insomnia, unhappiness, fear of death, syncopy, trembling and chill.

The condition in humans tends to effect more women than men, although a number of cases have been found amongst army recruits. There was inability to maintain muscular effort, an increased response to discomfort, and respiratory response at increased levels of CO_2 was greater. They showed a more ready response in pulse and respiratory rates to muscular movement, and utilised inspired oxygen less efficiently. Perhaps most significantly they developed a higher lactate level in the blood. The rise in lactate seen in these patients per unit of work were similar to those found in patients with nervous debilitating conditions such as arteriosclerotic and rheumatic heart disease (Pitts 1969). The conditions appear, in the first place, to be due to a continuously high liberation of adrenaline, which in turn chronically raised the blood lactate level.

Sodium Lactate

A phenomenon of considerable interest is that the symptoms can be duplicated by the infusion of sodium lactate, in susceptible patients. These infusions of sodium lactate cause, *inter alia*, tachycardia, and numbness and tingling of the skin. These symptoms are characteristic of a lack of calcium ions in the tissues. The symptoms lasted for 24 hours after infusion and in some cases for several days. If calcium is added to the sodium lactate, these symptoms do not appear, suggesting the lactate lowered the available calcium ions in the body.

These results suggest not only that intravenous sodium lactate (a sometime recommendation for conditions of capture stress) is harmful in that it tends to reduce ionised calcium, already in short supply, they also suggest that the effect of adrenaline is also to reduce calcium availability.

GLYCOGEN DEPLETION

Introduction

There is virtually no specific knowledge on the basic physiological mechanisms of muscle contraction in wild animals. We must therefore, at this stage, presume that the activation of muscle fibres and the depletion of muscle

glycogen in relation to the work intensity, follows a pattern which is very similar if not identical to that of man and the domestic animals.

It has been established (Chapter Twelve) that at maximum muscle activity, lactic acid is liberated at a rate and an amount that may induce a potentially lethal fall in pH. Conversely at sub-maximal effort, animals are capable of great stamina and prolonged periods of activity permitting ultimate survival. For example zebra were driven for approximately 30 km by helicopter in the Timbavati at sub-maximal speeds without apparent ill effects (own observations). It has been established also that a total effort or maximal muscle activity is more dangerous when the animal is rested, and less dangerous when nearing exhaustion; assuming for this argument that in the latter case, the output of muscular effort is indeed as great as in the former, i.e. that the animal is not saving itself unduly due to the actual exhaustion; a further saving will occur as a result of exhaustion of the muscle tissue reserves (Chapter Twelve).

It must also be assumed that at maximal and supra-maximal work loads there is no material utilisation of energy sources other than muscle glycogen, such as blood glucose, plasma free-fatty-acids or intramuscular lipids. It is probable that these alternative energy sources are utilised during prolonged low-intensity exercise with comparable difference in metabolic end products under conditions of hypoxia.

We are therefore dealing with muscle glycogen and with lactic acid as a product under predominantly anaerobic glycolysis. It would be valuable to know whether differing rates of glycolysis occur under varying conditions of exercise other than a proportionate increase in production of lactate in relation to increased inactivity, or whether a relatively small extra effort can result in a proportionately much greater production of glycolysis with concommitant lactic acid production and fall in pH.

Skeletal Muscle Fibres

It is known that human skeletal muscle contains fibres of two major kinds. These fibres may be distinguished on a histochemical basis according to differences in their myofibrillar ATPase activity (Edström and Nyström 1969, Gollnick, Armstrong, Saubert IV, Piehl and Saltin 1972). These two types of fibres have been designated as slow-twitch and fast-twitch respectively (Gollnick *et al* 1972). It has been pointed out by Barnard, Edgerton, Furuwaka and Peter (1971) that contractile speed is closely related to myosin ATPase activity so the two types of fibres are readily distinguished, the slow fibres being characterised by heavy staining for oxidative capacity, and slight staining for glycolytic capacity. Conversely, the fast fibres have a low oxidative and high glycolytic capacity.

The pattern of glycogen depletion in relation to differing degrees of exercise was examined by Gollnick, Piehl and

Saltin (1974), who noted that slow-twitch fibres were the first to become depleted of glycogen during submaximal exercise requiring loads of 60 to 80 percent of maximal oxygen uptake. Eventually, the fast-twitch fibres also became depleted of glycogen. When the work load was increased to 150 percent of maximal oxygen uptake, the initial glycogen depletion was in the fast-twitch fibres (Gollnick, Armstrong, Sembrowich, Shepherd and Saltin 1973).

These results suggest differing patterns of both oxygen and glycogen utilisation. Also differing groups of motor fibres containing predominantly slow- and fast-twitch fibres, the former being employed during relatively light exercise, and both being activated during heavy exercise.

Since the fast-twitch fibres have a low oxidative and high glycolytic capacity, they are heavily involved under these circumstances when there is a higher energy consumption per unit of tension. We can therefore expect a far greater liberation of glycogen under circumstances of maximal and supramaximal effort. At the same time, the liberation will be under low oxidative conditions, with the maximal reliance on glycogen as muscle fuel in the fibres with the high ATPase activity.

The experiments mentioned on human volunteers involved physical education students who were all presumably in a reasonable state of training and who performed daily exer-

cise. Comparison to the horse suggests that a resting animal is far more likely to develop pathological conditions associated with high rates of glycolysis than those involved in daily exercise; hence the condition in horses of azoturea or paralytic myoglobinurea, a spastic condition of the muscle fibres, develops during exercise after resting on a full diet.

Fear

The experiments mentioned here were all performed by volunteers under laboratory conditions and therefore do not involve another and probably important element, i.e. that of fear. It has been frequently observed that wild animals will chase others of the same species for long periods of time at apparent maximal effort without obvious detrimental results. Graded exercise under laboratory conditions and even aggressive intraspecific behaviour is unlikely to simulate the type of effort expended by animals subjected to effort under maximal adrenergic discharge. Neither is the exercise under controlled conditions comparable to maximal effort under field conditions in that only certain muscle groups are involved, as during cycling, while precipitate flight probably involves every muscle group in the body on a continuous basis and with maximal contraction resulting in high energy consumption which does not increase linearly with increased tension, but is nearer geometric function (Goldspink, Larson and Davies 1970).

PATHOLOGY AND POST MORTEM CHANGES

THE diagnosis of capture myopathy has rested largely on the appearance of areas of muscle fibres on autopsy. The muscle is pale with an altered appearance such as loss of cross striations. There is degeneration of the sarcolemmal sheaths.

This picture, however, is also similar to that of so-called pale, soft, exudative (PSE) pork which is essentially due to accelerated *post mortem* glycolysis in the musculature while the carcase is still warm after slaughter. The exudative condition rests on a poor water-binding ability of the carcase meat in turn due to protein degeneration present in the areas of pale muscle. This condition, in contrast to muscle dystrophy resulting on vitamin E deficiency, does not react to vitamin E and/or selenium therapy or prophylaxis in the living animal.

These facts are highly interesting in relation to true capture myopathy, especially in that the condition may occur without any muscle activity of the pig prior to slaughter and therefore are not a simple result of excessive muscular strain. The lesions in question may apparently be caused exclusively through the contraction of muscle in stress-susceptible pigs after or during slaughter. The causative factors appear to be active contraction of muscles just before or during death inducing a greatly accelerated glycolysis. The formation of lactic acid induces a fall in pH from

physiological levels to approximately 5,5 and this occurs normally over 12 hours. Under the conditions of sudden death this may take place within 30 or even within 10 minutes. The exceptionally rapid production of lactic acid under these conditions, while the carcase is still warm induced a protein degeneration manifested in a pale and soft meat. This, on histological examination, shows a typical degenerative appearance already described as occurring in both capture myopathy, and in selenium nutritional muscular dystrophy or true white muscle disease.

Particularly significant is the fact that the lesions are affected by the temperature of the carcase. Delay in evisceration causes exacerbation of the condition as does warm environmental temperature and consequent delay in loss of body heat after death. All these factors tend to obtain under field conditions.

Delay in loss of body heat, high body temperatures or raised temperatures during muscle contraction all tend to induce the pale muscle syndrome. This is in part due to the increased effect of lactic acid at high temperatures as mentioned above. But this is also due to a reduction of adenosine triple phosphate (ATP) used in muscle. Under normal conditions ATP is quickly resynthesised. Under conditions of anoxia such as that obtained during muscle contraction at the time of death or *pre mortem* conditions, ATP is not resynthesised. The attenuation of ATP levels at high temperatures and low pH alter the normal actin-

myosin filament structure of normal fibrils and formation of actinomycin (Lawrie 1966, Briskey 1964). This is largely the cause of the loss of cross striations usually mentioned as a typical phenomenon of capture myopathy. The contraction of the muscle is abnormal in that individual fibres tend to be more strongly contracted than others, inducing a distortion of the normal 'I' band structure. The sarcoplasmic structure becomes denaturated and is deposited on the structural proteins. There is a hydrolysis of the endomesium and loss of water-binding properties.

The lesions become established only at the time of death and no evidence of muscle degeneration can be obtained from biopsy of the living stress-susceptible animals. It is therefore evident that the following factors which are in fact found in a high proportion of pigs killed with a captive bolt pistol as compared to those stunned electrically (Dreyer, Naudé and Gouws 1972), have application to capture myopathy as diagnosed from necropsy. Assuming that these factors are not entirely restricted to pigs, the following may obtain under field conditions.

(a) The lesions may not be entirely due to the conditions pertaining at the time of death.

(b) Relatively small degrees of activity just prior to death may induce lesions apparently identical to those of true muscular dystrophy.

(c) The cooling of the carcase and time of evisceration have a marked effect on the incidence of muscle degeneration.

(d) The picture of degenerate muscle fibres may be induced without any overstraining in the commonly accepted sense of the word.

(e) Actual exhaustion with depletion of muscle glycogen causes a completely different *post mortem* picture to the pale muscle syndrome, the meat being dark and dry with a relatively high pH (Anonymous 1973).

Further investigation of these phenomena in relation to their possible implication in autopsies on captured wild animals may be valuable. Almost inevitably the body temperature of the carcase is raised, the environmental temperatures high, the carcase left unevisceratedand unhung while it is transported - often over several hours in sunshine in an open truck - to an area suitable for autopsy.

It is therefore apparent that reports of muscle degeneration based solely on the histological appearance of muscle fibres may be suspect, unless this is accompanied by a detailed report on the circumstances and manner of death, stating the environmental temperature, the *post mortem* handling of the carcase, and the interval between death and procurement of the tissue samples.

CHAPTER SEVENTEEN

CONCLUSIONS AND RECOMMENDATIONS

FORMS OF CAPTURE MYOPATHY

CAPTURE myopathy is a syndrome that shows differing aspects according to the type of stress experienced by the animal and its duration. These aspects are the following:-

(a) Hyperacute

(b) Acute

(c) Sub-acute

(d) Chronic or indefinite

The hyperacute and acute phases (a) and (b) appear to be associated with a profound acidaemia due to the liberation of metabolic acid into the blood stream. The rise in hydrogen ion concentration affects the respiration and the circulation. There is intensive dyspnoea resulting in hypocapnoea, but without the usual rise in pH normally resultant on a carbonic acid deficit. The heart rate becomes exceedingly rapid with inefficient heart action and a fall in systemic blood pressure. There tends to be a rise in pulmonary arterial pressure.

In the hyper-acute form the acidaemia is severe enough to cause cardiac arrest. Levels of blood pH recorded are sufficiently low in themselves to cause cardiac fibrilation

with resultant immediate circulatory collapse and death, the fall in blood pH is sufficient to account for cardiac arrest without additional pejorative factors. However, there is concommitantly a high blood potassium resultant on damage to somatic muscle fibres, and intravascular haemolysis, but also clinical indication of a maximal adrenaline discharge. Both of these factors are in themselves liable to cause acute death through cardiac fibrilation.

The acute form of capture myopathy likewise has a low blood pH as its principal causative factor. The hyperventilation on capture is mostly not able to lower the blood hydrogen ion concentration. The stressed animals tend to become more acidotic in spite of a condition of relative immobility. Skeletal musculature becomes stiff. Movement becomes difficult and apparently painful. The animal continues to exhibit a depressed appearance with high pulse and respiratory rates. Death with symptoms of pulmonary oedema occurred in about 12 hours or sooner in susceptible animals.

The sub-acute phase (c) is associated with the results of muscle and organ damage. The muscle damage manifests itself in contraction of the flexors resulting in knuckling over of the fetlock of some or all four legs. Frequently there is torticollis and there may be an inability to stand for which the term 'tetraplegia' has been used in reports describing this condition. Analysis of blood enzymes indicates damage to skeletal muscle and to major organs such as lung, liver, heart and kidneys. The animals may remain

in this condition for one to two weeks and die in spite of the application of a wide variety of treatments. There are high levels of blood metabolites. Autopsy shows necrosis in organs and muscle, and pigment deposits in the kidney tubules.

The indefinite phase or chronic debility (d) or delayed deaths due to the results of capture stresses are seen more rarely. Necropsy material shows fibrosed heart lesions and these appear to be the cause of deaths in apparently recovered and healthy animals when these are stressed by crating or similar manoeuvres a month or more after capture.

This is a very general classification for practical field purposes. One only of these forms, namely the acute, is amenable to specific treatment. The hyperacute form cannot be treated by definition as the animals die during the chase or in the immediate period of restraint. The third or subacute form may be treated symptomatically, and supportive therapy using B-complex vitamins such as thiamine is indicated, as is cortisone and vitamin E/ selenium therapy. The demonstration of renal blockage indicates the use of fluid therapy preferably of an alk-alising nature. The last form is cryptic and only gentle handling of all quarantine animals can be offered as a general recommendation.

DIAGNOSIS OF CAPTURE MYOPATHY

MOST of the parameters that indicate stress and incipient or established myopathy are not measurable under routine capture conditions. The animals should be exposed to the minimum of handling, and most techniques are both time-consuming and beyond both the technical ability and the available instrumentation of the ordinary capture team. Indicative parameters are the following:

ACIDAEMIA

THIS may be regarded as one of the most important indications for therapy. A low blood pH, especially if taken some minutes after capture when the breathing has stabilised is indicative of a metabolic acidosis. It has been shown that, even where the blood pH rises, the animals may still succumb, presumably due to damage from the low pH, particularly in areas where the tissue perfusion is defective and where the pH values may be lower than those of the venous blood. Serial samples preferably including capillary blood are indicated.

The clinical symptoms are tachycardia and dyspnoea and these may be used as an indication for the need of alkalising therapy, and both the technique of arterial puncture and the apparatus for anaerobic blood pH determination are impracticable for use under ordinary field conditions. Infusion should be given into a peripheral vein such as the recurrent tarsal at a rate of approximately 1 litre/10 minutes.

Lactate

Blood lactate levels are more easily established than blood pH and less sophisticated apparatus may be used. The samples may be stored if they are immediately deproteinised with ice-cold perchloric acid. It is doubtful, however, if the technique could be used on a general basis and is likely to be too time-consuming to be of practical value, so that reliance on ordinary clinical symptoms as described above is more likely to be of general use. The nature of the heart sounds on auscultation is a good guide to the state of the circulation as these become muffled and indistinct under conditions of cardiac incompetence and decreased cardiac output. Therapy for high lactate levels is as outlined for acidaemia.

Blood Enzymes

Blood enzyme determination (CPK, GOT, etc.) will give little help in assessing the stress condition from blood taken immediately after capture. Enzymes peak under most conditions after several days or a week, and the determinations during the peak period would be extremely valuable, but it is probable that the additional capture necessary for sampling at that time would be self-defeating, and may actually precipitate the capture myopathy condition. Enzyme levels after several weeks' captivity may, however, be a valuable indication whether the animals are fit to move, if these can be determined without inducing further stress. This will

depend on the species, and the type of enclosure. For example, eland that may be driven quietly into a crush, blindfolded and cast by Reuff's method, or tsessebe that may be similarly blindfolded and sampled standing may be tested without inducing a renewed rise in enzyme levels.

Cardiac Changes And Blood Pressure

The electrocardiogram and the cardiac output, indispensible in human clinical medicine, and the former in veterinary medicine, again have no place in the routine diagnosis of capture myopathy due to the prolonged restraint necessary for their application. The same applies to the measurement of the pulmonary artery, and systemic blood pressures. They are extremely useful, however, in determining which types of capture are stress-inducing. It is clear that physiological work has an important role to play in this context of animal capture, as all these four parameters have been shown in the course of this work to show major deviations from the normal and to be suitable indicators of stress and the advent of the myopathy syndrome.

Body Temperature

This is one parameter which is simple to determine in all animals captured by netting and other individual methods and may be determined in thirty seconds. Unfortunately one reading is of very limited value as animals with a very high initial rectal temperature reading (44,4 °C in

white bearded wildebeest *Connochaetes taurinus albojubatus* - own records) have survived. Conversely, animals with relatively low body temperature on capture may show a progressive increase during prolonged restraint to die of hyperthermia (Kariba - own records). A more rewarding exercise is therefore to make regular observations of environmental temperature, etc. and to relate these to the different species to compile records of their species susceptibility and resistance to the effects of exercise under warm conditions. An electric fan (run off the car battery) has been effectively used after wetting under experimental field conditions.

HAEMATOCRIT

THE capillary haematocrit value (as compared to central values) has been shown to be a most important indication of circulatory derangement. Unfortunately the capillary haematocrit alone is not meaningful, the same criterion being applicable to a lesser degree to capillary PO_2, PCO_2 and pH. The actual haematocrit value is theoretically a useful indication of the nutritional state, but not under capture conditions where adrenergic discharge may give grossly inflated values; the same may apply to animals that have been shot. Immobilised animals may show a drop in haematocrit values of about 20 percent during the first hour or so after capture.

Plasma Haemoglobin, Myoglobin And Potassium

THE presence of blood pigments in the plasma is highly indicative of exercise stress and foreshadows kidney complications. The quantity is readily subject to approximate quantitative determination using a small colorimeter with appropriate filter. This is one parameter whose routine determination may be undertaken. The results should be considered as applying to a group of animals rather than to individuals, and a useful criterion of the degree of stress imposed, e.g. distance run, speed, etc., in relation to the condition of the animals. Note also, that relatively minor stress such as manual restraint of sable antelope will induce discolouration of the plasma in 20 minutes or less. Duplicate samples should be taken to test for *in vitro* haemolysis. The time taken for centrifugation militates against this being used as a test to determine immediate treatment. Determination of blood potassium is an adjunct to this test, that may be usefully employed. Massive fluid therapy for all animals showing marked myoglobinaemia or intravascular haemolysis would be advantageous.

Blood Metabolites

DETERMINATION of creatinine, urea, etc. is a useful test in debilitated animals. High values may indicate protein breakdown, or impending kidney failure. Reduced kidney function predisposes to blockage of tubules. In these cases intravenous fluid therapy, especially in recumbent animals,

should be used, preferrably with an alkalising content. Too often debilitated animals refuse to drink and prostrate animals are left without shade or access to water. The fluid therapy may be combined with intravenous medication and nutrition.

PRE-CAPTURE NUTRITION

THERE are indications that many of the animals in the provincial reserves, routinely subject to capture, are in a poor state of health and nutrition, and are therefore a poor survival risk irrespective of the method of capture. Low haematocrit values of 20 percent were determined in eland captured at S.A. Lombard Nature Reserve, but which rose rapidly to 50 percent after relocation and artificial feeding. It is now shown (own records) that S.A. Lombard Nature Reserve is deficient in copper. Eland at Loskop Dam Nature Reserve are both anaemic and have a high incidence of blood parasites (2 percent - own records) and determination of trace elements show that most areas have seasonal low tissue values. Low selenium values in particular are likely to be conducive to capture myopathy. Particularly low values were determined for pregnant and lactating animals. Of thirteen female sable antelope recently captured, all eventually died, largely from dystokia probably associated with uterine inertia. The time of the year is clearly most important for animal capture. It is likely that the tradition of capture during the cold season

only needs modification. Hyperthermia is probably relatively unimportant if correct capture methods are employed, while low trace element levels and capture during the gestation period may be more deleterious.

An important aspect of capture stress clearly is the nutritional state of the animals prior to capture. An investigation of tissue trace elements for each reserve on a seasonal basis would be highly valuable, carcases being generally available from cropping or other sources. A valuable adjunct is the determination of serum proteins, especially albumin/globulin ratios at regular intervals throughout the year from animals immobilised for the purpose, together with detailed haematology, blood parasite load, etc. Work on these lines has commenced.

QUARANTINE

GENERAL figures (accurate figures not available) indicate that the greatest mortality in captured animals occurs in the holding enclosures. Some of this is no doubt due to capture myopathy. As the animals are frequently kept for long periods spanning many months, other factors must also be sought.

It is self-evident that research into the causes of this mortality should be undertaken as a first step to its reduction. The routine autopsy of carcases should indicate

the diseases which may be important factors particularly those brought on by stress. It is unlikely that the design of the holding enclosures (slatted rectangles for individual animals - 384 cm × 300 cm) could not be considerably improved.† Permanent quarters are conducive to massive tick-infestations and therefore to tick-borne diseases such as rickettsiosis, babesiosis, etc.

PREVENTION OF CAPTURE MYOPATHY

IN Chapter Fifteen it has been shown that the stress of capture may be greatly reduced if the animals are both exercised and familiarised with the capture routine. Work on both eland and black wildebeest has shown that the animals are readily accustomed to procedures that cause stress in the untrained animal.

Eland showed a remarkable difference between the first and subsequent runs on the track, the second run already resulting in negligible enzyme rises in spite of running at the identical speed and distance. Black wildebeest showed a reduced stress reaction after being run through part of the capture corral approximately twice monthly, with virtual elimination of the mortality experienced at the first run, and the original appreciable levels of plasma myo- and haemoglobin. The overall results from the two experiments i.e. the spectacular reduction in the enzymes indicative of capture stress in eland, the 50 percent reduction in mortality and elimination of visible plasma discolouration in the

† (e.g.) see Kakulas (1963) on myopathy in the captive rottnest quokka *Setonix brachyurus*.

black wildebeest, together with the known effects of exercise in horses (Chapter Fifteen) provides a strong indication of a saving effect from previous training on capture stress.

The training method is extremely simple. The animals may be exercised by men on foot, and usually readily run through parts of a capture corral, the wings only being first erected, with parts added piecemeal over a period of one to two months. The immediate practical advantage of the method is that corraling, usually considered impracticable in the Transvaal where the ground is stated to be too flat, can be used instead of nets with immediate saving of broken limbs, backs and necks, and avoidance of the considerable strain of struggling in the meshes and while the animals are being man-handled out of the net. Judging from the myoglobinaemia induced in paddocked sable by handling in a wooden crush (own records) the struggle in a net alone must induce near-lethal trauma and stress. There is in addition a marked reduction in both plasma pigments and enzymes indicating stress as a result of the preliminary exercise alone. The animals appear less frightened, and the speed of the chase (usually maximal to prevent animals breaking back past the trucks, carried out with much noise) can be considerably reduced. Once in the corral the animals can be immediately left to settle down thus further reducing fear and flight reaction. In Kenya, forty roan antelope were captured, in a large corral, and subsequently relocated for a distance of approximately 500 km (own records), and other

spectacularly successful capture exercises have been made, e.g. Hofmeyr (1974), Oelofse (1970). While conditions are not always comparable, all facts from such completely and spectacularly successful relocation exercises should be gleaned and compared with some of those in which 100 percent losses of valuable animals were sustained (such as the sable capture mentioned above, also the tsessebe relocation - see text) to try to establish the reasons for these disasters.

SUMMARY AND CONCLUSIONS

CAPTURE myopathy is a syndrome involving most of the body's functions, the circulation, acid-base balance, kidneys, and nervous system, in particular.

It is induced by intensive exercise in animals that are firstly untrained (thereby showing a similarity with paralytic myoglobinuria in the horse), and secondly pursued violently from a standing start, thereby inducing a massive anaerobic glycolysis.

The condition responds to therapy only in the immediately early stages when the acidaemia can be rectified. The blood and muscle pigment released from damaged muscle cells and blood corpuscles is likely to induce renal failure especially while the animals are (a) acidotic, (b) dehydrated and disinclined to feed or drink, and (c) subjected to continual

adrenergic discharge while under (unsuitable) captive conditions.

The causation of capture myopathy is largely unnecessary and can be avoided by prior training, familiarisation of capture technique, and especially the use of proper capture methods. Sudden chases over short distances at maximal speed (as advocated by most game departments) is a lethal procedure that should be abandoned. Maximal muscle strain as occurs in a net, is likely to be lethal especially to nervous animals such as sable antelope which destroys the muscle fibres within minutes of struggling against an unyielding object or manual restraint.

The nutritive state of the animal prior to capture is important. Greater knowledge should be acquired concerning the differences in our various reserves, and particularly the seasonal changes in body trace elements and serum protein content. The breeding season and pregnancy are factors, although the latter is probably of minor importance where capture methods are optimal. The capture season should be carefully established bearing these factors in mind rather than that solely of temperature, the latter being of reduced importance if the tempo of the chase is reduced, and the animals are corraled instead of manhandled.

The whole design and method whereby wild animals are held and quarantined should be investigated as well as the diseases

that are likely to and probably normally do cause heavy mortality. The massive tick burdens seen in animals in pens must be remedied and the possibility of disposable pens considered. Ideally animals should be subjected to the minimum of change and transport. Movement directly from the capture corral to the destination is vastly preferrable to the system whereby animals are moved from the capture area to individual pens and then to final destination as now obtains; in the first roan exercise referred to, the whole herd was left in the capture corral for almost six months before shipment, permitting gradual weaning onto artificial food (lucerne hay) under near natural conditions.

The possibility of capture by enticement rather than force should be investigated. The Transvaal Provincial reserves are probably ideal for inducing their animals to take limited quantities of artificially procured foods such as hay. This would have three immediate and spectacular benefits.

(a) The animals could be fed into corrals instead of stampeded into nets.

(b) The animals may be accustomed to the food eaten in captivity so that the correct ruminal flora for the new diet can be developed.

(c) The food given under captive conditions would be readily and immediately acceptable.

Other advantages are the possibility of supplementary feeding at the time of the year when (largely owing to lack

of pasture management) there is virtually no protein in the herbage, supplementation of possible mineral and trace element deficiencies, and an increased survival of calves due to improved nutritional state of the cows at the time of calving.

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These conclusions and recommendations take no account of the possible advantages that may be derived from feeding anxiolytics such as diazepam due to the fact that newly captured animals are likely to refuse food and water. A more sophisticated method of capture, however, based on prior artificial feeding, brings with it the additional bonus of possible administration of anxiolytics even before capture (such as spraying the hay or lucerne with molasses containing small amounts of suitable substances), during the early capture period and also before new moves are scheduled. Immediate experiments on these lines should be initiated.

An entirely new approach to the production of animals, their capture and relocation, is required if the present high wastage is to be reduced and the provincial restocking programmes are to be fully successful.

CHAPTER EIGHTEEN

EPILOGUE

On the basis of the work embodied in this report, and also as a result of the study of wild animal capture in a number of African countries, certain principles and guide-lines for the husbandry† of wild animals in the Transvaal can be put forward.

Concern at the high losses that occur in our wild animal production units at all levels from the low calf survivals to the high losses after relocation, has been expressed from time to time by all members of the Transvaal Division of Nature Conservation; both research and management staff alike.

It will therefore be counted as acceptable if the findings and recommendations of this dissertation on capture myopathy be expanded to cover general aspects of wild animal husbandry. On this basis it may be assumed that an improved management and capture policy could be evolved from an understanding of several principles which may be summarised as follows:

† For the rationale of the term 'husbandry' in the context of wild animal management for production, see Harthoorn (1969).

(a) That the animals in the Provincial reserves are not in a wild environment but restricted to relatively small enclosures by wire fences.

(b) That animals under these conditions are likely to be deficient in nutrients, susceptible to diseases, carry unduly large tick burdens, and are completely untrained with regard to exercise.

(c) That numerous ways and varieties of capture are available besides the heroic method of driving animals into nets by chasing with motor vehicles.

(d) That the stress of capture may be considerably alleviated by accustoming the animals both to the exercise and to the enclosures themselves.

(e) That immediately after capture, animals must be allowed to settle down with as little disturbance as possible, in surroundings as near as possible to their natural habitat.

(f) That ruminants are very susceptible to change of diet and that immediate change of diet alone may be lethal even without prior capture stress. Also that interruption of normal drinking habits may be highly detrimental under the circumstances, so that every effort must be made to leave the animals in as natural a state as possible with some natural food and water.

(g) That parasite burdens and disease are likely to increase as soon as wild animals are rendered static, so that, for instance, unless suitable measures can be taken to reduce ticks, all enclosures should be of a temporary nature and abandoned or destroyed after use.

(h) That stressed animals are very much more likely to die when stressed again during the so-called stress period, that is when enzyme levels are high. Considerable stress may be tolerated if the animals are left alone in suitable surroundings immediately after capture. Renewed stress of a further move, or the daily or hourly stress of human activity while in slatted enclosures without place to escape or hide is likely to precipitate a fatal stress or myopathy condition.

(i) That the optimal season for capture should be dictated by other criteria besides that of environmental temperature, and that the impact of the latter is likely to be greatly reduced by methods of capture as described in Chapter Fifteen. Other factors such as seasonal lows in tissue trace elements and gestation (although potentially also minimised) should also be taken into account.

(j) That research into veld management and tick control in our smaller reserves is overdue, and that the practice of sound methods to implement these is likely to pay high dividends, in the form of higher calf survival and reduced capture deaths.

Pretoria
May, 1976

SUMMARY

A total of 104 wild animals of nine species including sable, eland, blue and black wildebeest and tsessebe have been subjected to 60 000 tests during an investigation into the causes of mortality following mechanical capture. The work has been carried out primarily in three of the Transvaal Provincial nature reserves, but also in the Kruger National Park. Specimens for trace element work have been collected from the Hluhluwe/Umfolozi complex in Natal as well as from the Sabi Sand, Timbavati and Klaserie Game Reserves in the Eastern Transvaal.

The term capture myopathy has been selected as the most suitable term for the condition studied. Muscle lesions form the predominant necropsy findings while aberrations in functions of the body musculature resulting in stiffness, lameness and torticollis, form a predominating symptom.

Locomotory stress has been investigated in animals after normal capture, simulated capture, and in animals stressed under controlled conditions. Investigations have also been carried out on immobilised animals stressed with infusion of adrenaline. Normal or resting values have been established mainly on tamed animals.

Field techniques have been developed for the measurement of blood PO_2, PCO_2 and pH; glutamic oxaloacetic transaminase,

glutamic pyruvic transaminase, creatine phosphokinase and lactate dehydrogenase; also lactate, potassium, urea, blood urea nitrogen and creatinine. Electrophoresis was used to separate plasma myoglobin and haemoglobin; tissue selenium and other trace elements were investigated on a seasonal basis. Special techniques were worked out for the field catheterisation of the heart, pulmonary artery and aorta, and to measure the cardiac output.

An investigation of acid-base balance indicated a profound acidosis that developed rapidly in animals subjected to maximal exercise over a short distance. This acidosis was lethal in zebra and could be rectified by the infusion of bicarbonate solution at a rate of 1 000 m-equiv./litre of saline or balanced solution of ions for 250 kg body weight. Muscle pH was *significantly* lower than venous blood pH (t_8 = 7,203***) indicating a loss of tissue perfusion. There was a *significant* correlation between lactate and pH (r = -0,659**).

The electrocardiogram indicated cardiac changes associated with high blood potassium levels and low calcium ions. Heart rates up to 380 per minute developed in zebra, accompanied by dyspnoea, both of which were largely rectified by restoration of the blood pH. During this stage there was a sharp drop in cardiac output due mainly to the effect of low pH on cardiac muscle.

Systemic blood pressure showed a fall that manifested partial correlation with the lapse of time after exercise ($t_{23.1}$ =

0,4287) associated in some animals with increasing acidaemia. There was a rise in pulmonary artery blood pressure to double accepted normal values. The pulmonary hypertension was modified but not eliminated by raising the blood pH to a physiological range.

There was a *significant* correlation of increase in body temperature with exercise, the higher body temperature (r = 0,7444***, t_7 = 4,610***) being found in animals exercised over a short distance.

Body temperature of most species fell spontaneously and there was little evidence of malignant hyperthermia except in sable antelope after immobilisation, with adrenaline infusion when artificial cooling was necessary. There was a *significant* correlation between hyperpnoea and high speed (r = 0,9739**). High haematocrit values in blood taken from capillaries, together with low PO_2, pH and high PCO_2 indicated stasis in peripheral blood vessels. A rise in the haematocrit of central blood after infusion with a fall in central PO_2 indicated a return of sequestrated blood to the circulation.

High values of CPK, etc. were seen in all animals subjected to locomotory stress (except in trained animals - see below), and enzyme values as well as the presence of blood pigments have been taken as the principal criteria of stress in animals that could not be subjected to more extensive physiological tests such as electrocardiogram readings.

Plasma myoglobin and haemoglobin showed a *significant* correlation with distance run before capture (haemoglobin: r = 0,8080***); myoglobin: r = 0,7850***). There was a *significant* rise in blood potassium (r = 0,935***) to near lethal levels. Reduced kidney function as assessed by the rise in blood metabolites and the prevailing acid conditions predispose to blocked tubules due to myo- and haemoglobin as determined at autopsy of animals that had died with symptoms of capture myopathy.

Blood metabolites such as creatinine, urea and blood urea nitrogen showed a rise after exercise and after adrenaline infusion. There was a *significant* difference in these before and after (males: t_3 = 3,298***; females: t_3 = 3,463***) exercise as well as between male and female eland (males: t = 6,0***; females: t = 5,619***). Alpha blockade with phenoxybenzamine hydrochloride tended to induce a drop in creatinine, etc., and an increase in the cardiac output. An increase in kidney function as a result of this treatment was not indisputably demonstrated.

Seventeen trace element contents from liver collected from animals cropped for reasons unconnected with this work were investigated. There was a *significant* correlation of variations in five elements, namely, chlorine, cobalt, magnesium, sodium and zinc on a seasonal basis (r = 0,9540*** etc.)

Seasonal differences in selenium and other important trace

elements such as copper exceeded 200 percent. Particularly low levels of selenium were found in lactating and prenant animals.

Attempted therapy using selenium and vitamin E, B-complex vitamins and hydrocortisone appeared to have a saving effect but was not very significant statistically, probably due to the small numbers of animals used. Bicarbonate therapy after acute stress showed a hundred percent saving effect, but is not regarded as generally practicable due to the additional handling of animals this would entail and the difficulty of intravenous infusion under most field conditions. Differences seen in enzyme peaks in animals between the first and subsequent runs were *highly significant* (CPK: t_3 = -14,101***, etc.), and groups of animals could not be used at monthly intervals owing to the difference between the first and subsequent stress reactions to exercise in spite of standard speed and distance.

Differences were also seen in plasma myo- and haemoglobin between animals run for the first time, and after several dummy or training runs at low speed using only part (mainly the wings) of the capture corrals.

These and other observations on the changes that occur in reactions to stress by animals even after very moderate exposure to training and exercise supports the conclusion that preliminary exercise of animals and their familiarisation to capture methods and corrals is the best prevention of capture myopathy.

OPSOMMING

DIE oorsake van mortaliteit in wilde diere wat gepaard gaan met verskillende meganiese vangmetodes is nagevors. Sowat 60 000 toetse is uitgevoer op bloed- en ander monsters van altesaam 104 wilde diere van nege verskillende soorte. Die proefdiere sluit in die swartwitpens, eland, die blou- en swartwildebees en die basterhartbees. Die navorsingswerk is uitgevoer in die Nasionale Krugerwildtuin sowel as in drie van die grootste Provinsiale natuurreservate in die Transvaal. Weefselmonsters vir spoorelementontledings is onder andere versamel in die Hluhluwe/Umfolozi-wildtuinkompleks in Natal sowel as in Sabi Sand, Timbavati- en Klaseriewildtuine in die oos-Transvaal.

Die siektetoestand wat veral spesiale behandeling ontvang het in die ondersoek staan onder andere bekend as vangmiopatie (Capture myopathy). Die siektetoestand is daarvoor verantwoordelik dat die spierstelsel aangetas raak en in die lewendige wilde dier word vangmiopatie gewoonlik gekenmerk deur styfheid, verswakking en/of verdraaing van die nek. By nadoodse ondersoek is baie uitgesproke spierletsels gewoonlik sigbaar.

Spanningstoestande is in wilde diere bestudeer nadat dit teweeg gebring is deur normale of nagebootste vangmetodes. Proefdiere is ook onder gekontroleerde eksperimentele toestande aan spanning blootgestel en die toestand van spanning

is verder kunsmatig teweeggebring deur die infusie van adrenalien. By wyse van vergelyking is fisiologiese ondersoeke gedoen op makgemaakte wilde diere. Monsters van dié diere is vir dieselfde doel ontleed.

Moderne ontledingstegnieke is ontwikkel of aangepas om onder primitiewe veldtoestande die volgende ontledings te kan doen: Bloed PO_2, PCO_2, pH, glutamien oksaalasyntransaminase, glutamien pirodruiwetransaminase, kreatienfosfokinase, laktaat dehidrogenase, asook laktaat, kalium, ureum, ureumstikstof en kreatinien. Daar was voorts ook van elektroforesetegniek gebruik gemaak om plasmamioglobien van hemoglobien te onderskei. Die seisoenale spoorelementprofiel in diereweefsels is ook ontleed en bestudeer. Spesiale tegnieke is voorts ook ontwikkel volgens hulle veldtoestande die hart, longslagaar en aorta met kateters te penetreer om sodoende die hartfunksie van wilde diere te kan bestudeer.

Hierdie ondersoeke het aan die lig gebring dat wilde diere wat oor 'n kort afstand aan uitermatige oefening blootgestel word 'n noemenswaardige versuring van die bloed ondergaan. Asidose was onder andere vir mortaliteite in ooreisde sebras verantwoordelik. Die fisiologies versteuring kan kunsmatig reggestel word deur die dier te behandel met 'n oplossing van natriumbikarbonaat. Gunstige resultate is verkry deur die binne-aarse toediening van sowat 1 000 m-ekwivalent natriumbikarbonaat per liter gebalanseerde soutoplossing per 250 kg dieremassa. Na ooreising was

die suurtegraad van die spiere *betekenisvol* laer as dié van die aar-bloed (t_8 = 7,203***). Die bevinding dui vanself-sprekend op verswakte vloeistofwisseling in die dierweefsels. Daar was voorts 'n *betekenisvolle* korrelasie tussen laktaat en suurtegraad (r = -0,659**).

Elektrokardiografiese ondersoeke het daarop gedui dat 'n verhoging in the bloedkaliumwaardes en gepaardgaande verlaging in die kalsiumvlak gevolg word deur versteurings in hartfunksie. Versnellings van die hartspoed na 380 slae per minuut wat in die sebra gepaard gegaan het met asemhalingsnood, is kunsmatig reggestel deur die verhoging van die suurtegraad van die bloed. In behandeling was asidose deurgaans daarvoor verantwoordelik dat die verswakte hartspier doeltreffend gefunksioneer het en kleiner hoeveelhede bloed per slag gelewer het.

Die verlaging in sistemiese bloeddruk kon tot 'n mate gekorreleer word, in die tydverloop na blootstelling aan oefening ($t_{23.1}$ = 0,4287), by party diere was die verlaging in bloeddruk geassosieer met die ontwikkeling van bloedsuurheid. Die verhoging in bloeddruk in die longslagaar was uitermatig en het somtyds twee keer die normale waardes oortref. Die kunsmatige verhoging van die suurtegraad van die bloed het hierdie toestand tot 'n mate beïnvloed maar nie geheel en al reggestel nie.

Daar was 'n *betekenisvolle* korrelasie tussen die ooreisde dier se liggaamstemperatuur en die mate van oefening waar-

aan die diere blootgestel is. Liggaamstemperatuur was die hoogste (r = 0,7444***, t_7 = 4,610***) in diere wat vinning oor kort afstande moes hardloop.

Na oefening het die liggaamstemperatuur van meeste diersoorte vanself na die normale teruggekeer. In die meeste gevalle was daar geen kwaadaardige hipertermie nie. Uitsonderings het wel by die swartwitpens voorgekom nadat die diere wat met chemiese middels gevang is met adrenalien behandel is om 'n verlaging in die liggaamstemperatuur kunsmatig teweeg te bring. Daar was voorts 'n *betekenisvolle* korrelasie tussen asemhalingstempo en die spoed waarteen die diere gehardloop het (r = 0,9739**). Hoë hematokriet waardes in kapillêre bloed tesame met lae PO_2, pH en hoë PCO_2 het op periferale stase gedui. 'n Toename in die hematokrietwaardes van bloedmonsters uit die sentrale sisteem versamel het daarop gedui dat die fisiologiese skoktoestand suksesvol deur middel van behandeling reggestel is.

In alle proefdiere wat aan oormatige oefening blootgestel is, was hoë CPK en ander waardes waargeneem en 'n studie van die ensiemvlakke sowel as 'n ondersoek na die voorkoms van bloedpigmente is as die belangrikste maatstawwe van ooreissing in proefdiere aanvaar wat nie aan meer intensiewe ondersoeke onderwerp kon word nie.

Plasmamioglobien en hemoglobien kon *betekenisvol* gekorreleer word met die afstand wat die diere voor vangs afgelê het

(hemoglobien: r = 0,8080***, mioglobien: r = 0,7850). Daar was ook gevalle, lewensgevaarlik, toename in bloedkalium (r = 0,935***). Bloedontledings het op swak nierfunksie gedui en dit, tesame met die toestand van asidose, het daartoe bygedra dat die nierbuisies deur mioglobien en hemoglobien geblokkeer is. Die ontwikkeling van dié toestand is deur nadoodse ondersoeke bevestig.

Bloedmetaboliete soos kreatinien, ureum en ureumstikstof het merkbaar gestyg na oefening en na adrenalieninfusie. Daar was 'n *betekenisvolle* verskil hier voor en na oefening (bulle: t_3 = 3,298***, koeie: t_3 = 3,463***) asook tussen elandkoeie en bulle (bulle: t = 6,0***, koeie: t = 5,619***). Alfablokering met fenoksiebensamien hidrochloried het geneig om 'n daling in kreatinien, ens., te veroorsaak asook 'n verhoging in die hartslagvolume. 'n Verhoging in nierfunksie as gevolg van hierdie behandeling was nie onteenseglik bewys nie.

Seventien spoorelementinhoude van lewers is ondersoek. Hierdie lewers is gekry van diere wat geskiet is vir ander redes, en nie verband hou met hierdie werk nie. Daar was 'n *betekenisvolle* korrelasie van variasies van vyf elemente, naamlik, chloor, kobalt, magnesium, natrium, en sink op 'n seisoensbasis (r = 0,9540***, ens.). Seisoensverskille in selenium en ander belangrike spoorelemente soos koper, het 200 persent oorskry. Besondere lae vlakke van selenium is gevind in lakterende en dragtige diere.

Die toediening van selenium, vitamien E, B-kompleksvitamiene en hidrokortisoon het gedui op 'n beskermde effek, maar dit was nie statisties betekenisvol nie, waarskynlik as gevolg van die klein getalle diere wat gebruik is. Bikarbonaat-terapie na uitermatige spanning het 'n volkome beskermende effek gehad, maar dit word nie as 'n algemene praktiese metode aanvaar nie omdat dit die addisionele hanteering van diere inhou, asook omdat die binne-aarsinfusie onder veld kondisies baie moeilik is. Die verskille in ensiemspits waargeneem in diere tussen die eerste en opvolgende oefenlope was *hoogs betekenisvol* (CPK: $t_3 = -14{,}101^{***}$, ens.), en die groepe diere kon nie met maandelikse-intervalle gebruik word nie, weens die verskil tussen die eerste en opvolgende spanningsreaksies na die oefening. Hierdie het gebeur ten spyte van standardssnelhede en afstande afgelê.

Verskille is ook waargeneem in plasmamio- en hemoglobien van diere wat vir die eerste keer gehardloop het, en dié wat na skeie oefenlope teen 'n lae spoed in die buitensgedeeltes van die vangkrale gehardloop het.

Hierdie, en ander waarnemings op die verandering in reaksie van diere teen oorspanningstoestande na selfs 'n ligte graad van oefening, dui daarop dat die voorkoming van vangmiopatie gesete is in die voorafoefen en gewoonmaak van diere aan vangmetodes en vanghokke is.

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APPENDIX A

DRUG DOSAGES FOR IMMOBILISATION

Dosages for adult (from 12 months) tsessebe, sable antelope and black wildebeest

COMPOUND	CONCENTRATION	TOTAL DOSE	DOSE PER BODY WEIGHT
fentanyl citrate (narcotic and principal immobilising drug)	100 mg/ml	30 mg or 0,3 ml	18 mg/100 kg or 0,2 ml/100 kg
xylazine hydrochloride (adjuvant and synergist)	20 mg/ml	10 mg or 0,5 ml	6 mg/100 kg or 0,33 ml/100 kg
azaperone (tranquilliser)	200 mg/ml	150 mg or 0,75 ml	100 mg/100 kg or 0,5 ml/100 kg
atropine sulphate (if needed - parasympatholytic)	10 mg/ml	15 mg or 1,5 ml	10 mg/100 kg or 1 ml/100 kg
nalorphine hydrobromide (antagonist)	20 mg/ml	100 mg or 4 ml	62 mg/100 kg or 3 ml/100 kg

APPENDIX B

FORM USED FOR FIELD WORK

(and a typical example of its use)

DATE 21/3/74

SPECIES Zebra	PLACE CAUGHT Lindanda - K.N.P.
AGE 2 years	DISTANCE CHASED (km) 2 km
SEX ♀	AVERAGE SPEED CHASED (km/h) 30,0
WEIGHT (kg) ± 220	TIME TAKEN TO CHASE 4 mins / TIME CAUGHT 9.32 a.m.

TIME	BLOOD SAMPLES ART.	M.V.	CAP.	VEN.	ENV. TEMP. WET	DRY	PHYSIOL. PARAM. HEART	RESP.	TEMP.	REMARKS
9.34					21	27	180	40	39,1	
9.36		✓								local anaesth.
9.37				✓						
9.48	✓									
9.54			✓							forced resp.
10.02					21	28	152	56	39,4	
10,04	✓	✓								
10,06				✓						heavy breathing
10,14			✓							sweating
10.23	✓	✓								
10.25					21,5	27,5	132	60	39,5	
10,32			✓							
10.33				✓						
10.40										start infusion
10.44										end of 500 ml inf.
10.45										minimal improvement - kicking
10.47					21,5	27,5	128	36	39,3	
10,49	✓									
10,50		✓								
10.54			✓							
10.56										start of 2nd half inf.
11.02										end of 1000 ml inf.
11.03										further improvement
11.06					22,5	28,5	128	32	39,2	struggling + kicking
11.07	✓	✓								
11.13			✓							resp - less forced - more normal
11.19				✓						struggling - active
11.22										released - stiff legged

APPENDIX C

CAPTURE MYOPATHY IN TWO NYALA

A visit was made to Hluhluwe Game Reserve to examine two male nyala which were reported to be recumbent.

One of these, a young male had been sick since capture. It exhibited typical symptoms of capture myopathy - stiffness of the limbs, knuckling over at the fetlocks and general malaise.

The second male, a sub-adult, had been sick since it had been involved in a fight twelve days previously.

The enzyme picture of the first animal only was characteristic of capture stress with a high level of creatine phosphokinase (823 mU/ml) indicating damage to skeletal muscle and a very high level of lactate dehydrogenase (916 mU/ml) indicating damage to the cardio-vascular system. The glutamic pyruvic transaminase level was also raised (180 mU/ml).

Both animals died in the course of the following week. No therapy was attempted.