GIDDINGS S R

WATER METABOLISM IN THE GEMSBOK

ORYX GAZELLA (LINNAEUS)

MSc(Zoology)   UP   1990

Water metabolism in the gemsbok <u>Oryx</u> <u>gazella</u> (Linnaeus)

by

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ABSTRACT

This study aimed to identify some of the physiological mechanisms that enable gemsbok to live in regions where drinking water may be saline or non-existent. The response of captive male gemsbok to fresh and 1,8% saline drinking water and dehydration was assessed seasonally by the measurement of body fluid compartments, renal function, some blood variables, food and water intake, energy assimilation and faecal variables. Fewer significant differences in the variables measured were found in animals acclimated to saline water than in those that were dehydrated. Seasonality also influenced the physiology of the species. Gemsbok were more

tolerant of dehydration than many other species and in this context may be considered arid adapted. It is concluded that 1,8% saline drinking water is less stressful than dehydration.

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" The oryx is as aggressive as the eland is tractable.
  It wields its rapier like horns with great facility -
  those who study its physiology get physical as well
  as mental exercise "

C. R. Taylor.

1969.

TO MY PARENTS

CHAPTER 1

GENERAL INTRODUCTION

INTRODUCTION

The Kalahari

The Kalahari Gemsbok National Park (KGNP), comprising 9591 km² is situated between 20 ° and 20 ° 53′ E and 24 ° 45′ and 26 ° 30′ S. This arid area (Leistner 1967), commonly referred to as the Southern Kalahari (Fig. 1.), is the driest part of the Kalahari ecosystem receiving 249 mm of rain on average each summer (Mills 1977). However, this is highly erratic, and precipitation is frequently much less (Werger 1978). The region is also characterized by high ambient summer temperatures and radiation from October to April, and Kriel (1967, in Van Rooyen 1984) reports that evaporation exceeds rainfall, indicating a water debt. Winter, from late May to August, is typified by warm days and cold nights.

The gemsbok

Five subspecies of <u>Oryx gazella</u> have been described by Ansell (1972). Of these, only <u>O. gazella gazella</u>, the gemsbok, occurs in Southern Africa. Du Plessis (1969) has noted that their

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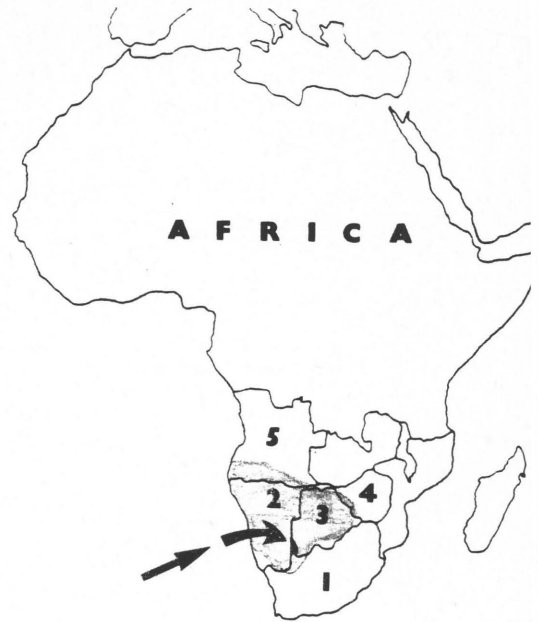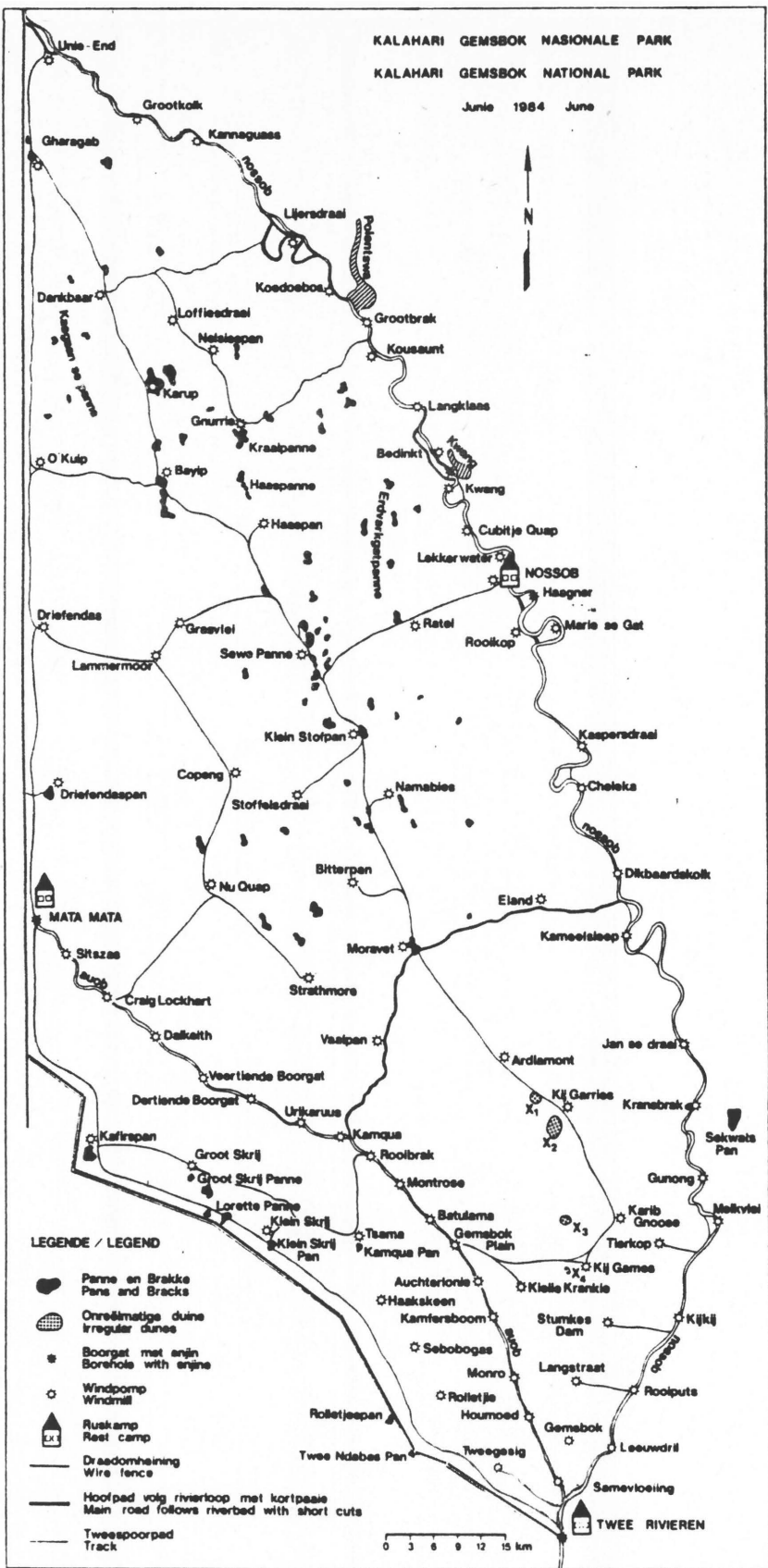
Compiled by J. du P. Bothma and drawn by Amanda Nel.

Fig. 1. Distribution of <u>Oryx</u> <u>gazella</u> <u>gazella</u> (shaded area) with reference to the Kalahari Gemsbok National Park to show location and number of artificial water holes (map from <u>Koedoe</u> (<u>Suppl</u>.) 27, 1984)

distribution is confined to more open arid regions including parts of Angola, Namibia, Botswana, eastern Zimbabwe and the northern Cape province (Fig. 1.). Furthermore, Du Plessis (1969) noted that the species originally inhabited the area south of the Orange river, even extending as far south as the Karroo, but the largest population of gemsbok is to be found within the KGNP (Dieckmann 1980).

Gemsbok are grazers but they may browse, especially when the water content of the grass is low (Knight <u>pers. comm.</u>). In addition, when other sources of water are deficient, gemsbok make much use of plant underground storage organs (Williamson 1987). It is known that territorial males may remain for extended periods of time in regions devoid of surface water for drinking (Knight <u>pers. comm.</u>).

Gemsbok are gregarious, and although adult males are territorial, they will accompany both mixed herds and nursery herds (Smithers 1983). Herd sizes may range from two to over fifty individuals, although it is more common to find ten to twenty individuals in a herd.

Gemsbok are aseasonal breeders (Skinner, Van Zyl & Oates 1974), and give birth after a gestation period of 264 days (Brand 1963). The calf is rarely seen until it is a few weeks old, when it will join

M.H.Knight, National Parks Board of Trustees, Pretoria, South Africa.

either a mixed or nursery herd (Dieckmann 1980). Little is known of the physiology of this sub-species, although much work has been done in East Africa on the Beisa oryx, <u>Oryx gazella beisa</u>, by Taylor (1968, 1969, 1970a and 1970b). The ability of the gemsbok to survive in arid regions is evident, yet how this is achieved still needs to be understood.

The project

More than eighty boreholes supply surface water throughout the KGNP. Over half pump saline water of greater than 1,8% total mineral concentration, the major ions being sodium (Na), chloride (Cl) and sulphate (SO_4) (Smit 1964). Due to the high salinity of the water, the necessity of such boreholes for providing drinking water has been questioned. On one hand Child, Parris & Le Riche (1979) found that animals made regular use of the mineral water provided, and they suggested that this may increase animal numbers in the region. On the other hand Mills & Retief (1984) found that only blue wildebeest (<u>Connochaetes taurinus</u>) were favoured by the provision of artificial watering points, with gemsbok being independent of water throughout the year.

Although the question of whether gemsbok need to drink appears to have been answered by Mills & Retief (1984), observations have shown that gemsbok do drink which implies that, although they may not need to drink, they will drink if water is available. In times of drought they should drink more frequently since water in herbage

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is reduced as dicotyledons themselves respond to aridity by reducing water content and shedding leaves, while monocotyledons lignify (Skinner, pers. comm.). In addition, less metabolic water becomes available as animals use up their fat reserves. Under such conditions, could gemsbok survive drinking saline water? Furthermore, would the drinking of saline water be more beneficial than possible dehydration?

The present study was aimed at finding the answers to these questions.

MATERIALS AND METHODS

Costs involved in a study of this nature limited the number of study animals to five. Furthermore, the number of experiments that could be replicated within a calendar year was limited. Statistical problems associated with small sample sizes were recognized, and therefore it was decided to utilize one salinity for repeated sampling in both summer and winter. The salinity chosen to be tested against fresh water, was 1,8%, based on Dreyer's (1987) observations that gemsbok did not tolerate water containing more than 1,8% saline. Since it was impractical to provide naturally occurring water of this concentration, saline drinking water was made by the addition of 1,5 g of salt per 100 ml of tap water,

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which was used as the fresh water control, and which contained 0,3% salts. The salt used was supplied commercially and consisted of 95% sodium chloride (NaCl), 4,5% sulphates and 0,5% other ions.

Experimental animals

<u>Capture</u>

In December 1987 five wild sub-adult (6 - 12 month old) male gemsbok were captured. Each animal was darted with approximately 0,025 mg/kg etorphine hydrochloride (M-99, Reckitt, Durban, S.A.) and approximately 0,08 mg/kg xylazine (Rompun, Bayer, Leverkuesen, Germany) (see below for description of drugs) using a dart gun (Telinject, Randburg, S.A.), and transported to Nossob Rest Camp, where individual holding pens had been constructed.

Before being revived, body measurements were taken, and the horns were covered with plastic piping as a protective measure. Each animal was injected intramuscularly with 2ml Trilafon Enanthate (Scherag, Isando, S.A.). This drug, a long acting tranquilizer intended for the treatment of human nervous disorders, was also found to be useful as a long acting tranquilizer in medium sized ungulates (Marais <u>pers. comm.</u>).

<u>Care</u>

The holding pens

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Each of the pens constructed at Nossob Camp consisted of a small roofed enclosure and a larger open area (50 sq. metres). The retaining walls were made of heavy duty wire fencing and bamboo shafts were used to delineate the wire, prevent entanglement of the animals extremities and conceal the animals from each other. The roofed enclosure had a sloping concrete floor with drainage facilities to collect urine, and also to prevent loss of faecal material. During experimental procedures, animals could be penned within the enclosure to ensure urine and faecal collection. Within the enclosed area were the food and water troughs, and food spillage from the trough could be determined by collection of spilled food from the concrete floor. The water trough consisted of a 50 l drum half filled with water. This effectively eliminated spillage due to drinking or movement of the container.

All pens were linked to each other by a corridor which ended at a cattle crush. The moving crush was built around a scale accurate to one kilogram.

Management

For the first two weeks, grasses were collected in the field and provided ad lib. There was no sign of food rejection by any of the animals and during this time dried lucerne was gradually added in increasing proportions. All animals later fed readily on pure milled lucerne. A dietary supplement consisting of drought pellets (Veekos Co-Op, Upington, S.A.) was given to the animals when not on

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experimental protocols (nutritional values for both feeds are presented in Table 1.). Fresh water was also provided ad lib. to all animals. Animals were acclimated under these conditions for three months. Holding pens were cleared of faeces once every three days and the floor was washed once weekly. Following this period, animals were kept under these conditions when not on experimental protocols.

During the duration of the experiments (15 months) two animals died, one of a neck injury, cause unknown. A post-mortem investigation revealed damage to the neck musculature. Moreover, rumen activity was found to have ceased some time previous to this as judged by the dry appearance of undigested rumen contents. The intestine was empty. It was suggested that the nerve tissue leading to the rumen, which passes through the neck musculature, had been damaged (Hofmann, pers. comm.)

This animal was replaced by a slightly older, larger and heavier individual. However the number of experimental animals was again reduced to four shortly afterwards due to another death. This animal, on a Dehydration trial (discussed below), lost condition rapidly in winter. During the experiment, night time temperatures were consistently below -4°C, and it is believed that this together with a decline in body reserves, lead to the contraction of

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Table 1. Composition of feeds provided to gemsbok during the study.

Constituent (%)	Lucerne	Drought pellets
Crude protein	12 - 15	10 - 11 (minimum)
Fat	7 - 10	2,5
Crude fibre	18 - 25	20 (maximum)
Moisture	6 - 9	12
Urea	not analysed	0,5 (maximum)

(Drought pellets analysed by Veekos Co-Op, Upington, S.A.)

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pneumonia, and finally death. Once symptoms were noted, the animal was immediately treated but this was too late to prevent death. A post-mortem investigation revealed numerous dark patches within the lungs.

<u>Release</u>

Prior to their release, animals were fed grasses for two weeks. When released they were darted with etorphine hydrochloride and xylazine, weighed, measured and ear-tagged. They were then transported into the wild and released after being revived (discussed below). The animals were observed grazing two days after being released.

Experimental design

The study was divided into a summer component and a winter component with repetitive trials being carried out in each season. Although summer has been broadly defined as occurring between October and April, with winter occurring between May and August, a more detailed classification based on maximum and minimum temperatures, was used to define season. Summer was defined as occurring when the average daily maximum temperature was greater than 28°C and the average daily minimum was above 10°C. Winter was defined to occur below these values.

Trials consisted of either providing the animals with drinking water <u>ad</u> <u>lib.</u> (Hydration trial), or removing all drinking water

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(Dehydration trial). Two drinking water regimes consisting of either fresh water or saline water of 1,8% concentration were provided. Water was changed at least once every two days, usually each day, to ensure concentration of dissolved ions due to evaporation did not occur.

In the Hydration trials, an acclimation phase, an experimental phase, and a recovery phase were scheduled. When drinking water was removed during the Dehydration trials, there was no acclimation phase and only experimental and recovery phases were involved. Food was supplied <u>ad</u> <u>lib.</u> throughout all phases and replenished twice daily at 09h00 and 16h00.

During the acclimation phase, two animals were given 14 days to acclimate to one of the two drinking water regimes, while the remaining two animals acclimated to the other water regime. Two trials were conducted with five animals, and in these trials, two animals were acclimated to one water treatment, and three to the other. Animals were darted and anaesthetized on Day 1 of each acclimation phase, following which they were weighed, and then revived. Fourteen days were considered adequate for acclimation to a water regime. Food and water consumption and climatic data were recorded daily during this period.

The experimental phase which followed acclimation began on Day 15 and lasted until Day 20. Water and food intakes as well as faecal

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output were measured daily. In the Dehydration trials, the experimental phase began on Day 1 when water was removed. The experiment terminated on Day 5. During this time food intake, faecal output and climatic data were recorded daily.

On Day 15 and Day 20 in the Hydration trials, and on Day 1 and Day 5 in the Dehydration trials, various other variables were measured following anaesthesia. The variables measured and their specific methods and results form the basis of the chapters to follow. Only methods common to all variables measured will be presented in this chapter.

Following the experimental phase, a recovery phase was allowed in order to ensure that the effects of the previous treatment did not interfere with the following one. Fourteen days with access to ad lib. fresh water and food were found to restore weight losses and apparent condition. However this period was invariably extended to three weeks following the Dehydration trials, since body condition did not recover to previous levels within the shorter time period.

Following recovery, animals were placed on another trial and randomly assigned to a drinking water regime if it was a Hydration trial, or water restriction if a Dehydration trial was planned. While there were nine Hydration trials during the study, only three Dehydration trials were carried out. This was due to the fact that the Dehydration trials were more debilitating. Moreover, it was not

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possible to weigh the dehydrating animals to ascertain the extent of the stress applied since then anaesthesia was required. After due consideration, this was only considered as feasible once every three days. Since fatality was estimated to occur after a weight loss of 15 - 20% (Ingram & Mount 1974), it was crucial to determine daily weight losses. This could only be done by visual subjective assessment, and fewer trials were carried out in order to ensure fatality did not occur. Furthermore, all these trials were carried out in summer since one animal died as a result of loss of condition in winter.

Experimental procedure

The use of drugs

The use of anaesthesia to obtain blood samples was not envisaged in the planning of the study. It was assumed that following three months of captivity and training the animals would stand passively in the crush and allow sampling to be carried out. This was not the case. Even after 15 months in captivity the animals were aggressive and dangerous when handled. Furthermore, restraining an animal in the crush proved traumatic and introduced an element of stress. Only one animal was successfully placed in the crush without anaesthesia, although it was highly stressed by the procedure.

Although known to influence physiological variables in gemsbok (Fourie & Van Ouwerkerk 1984), anaesthesia was the only feasible means of restraining these animals. Moreover, even though it is

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possible that physiological variables have been altered by anaesthesia, this alteration is likely to be more constant than that of stress, since a similar amount of anaesthetic was used each time.

The drugs used were etorphine hydrochloride and xylazine, marketed as M-99 and Rompun, respectively, and carfentanil (Janssen Pharmaceutica, Johannesburg, S.A.). M-99 is a powerful analgesic derived from thebaine, an opium alkaloid (Harthoorn 1973). It has a similar pharmacological action to morphine, which includes respiratory depression. Tachycardia and increased blood pressure may also result.

The unavailability of M-99 resulted in another drug being used for some of the experiments. This drug was carfentanil, a penthidine analogue (Harthoorn 1973). This drug is similar to fentanyl (Janssen Pharmeceutica, Johannesburg, S.A.) except for the fact that it is more potent (Scheepers, pers. comm.). Its action is similar to morphine although there is less respiratory depression (Harthoorn 1973). The effects of this drug on the physiological variables measured could not be distinguished from those following M-99 administration. Renal function was thought to be the variable most prone to the effects of anaesthesia, but there were no significant differences between the values obtained with either

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drug. Rompun, a centrally acting muscle relaxant, augments the condition of sleep and of analgesia in ruminants (Pack leaflet information). Its effects on the physiology of the animal include transient increases and decreases in blood pressure and a decreased heart rate. Whenever captive animals were darted, the following approximate dosages and drugs were used : 0,025 mg/kg M-99, 0,094 mg/kg Rompun and approximately 2 ml sterile water or 0,008 mg/kg carfentanil and 0,094 mg/kg Rompun with 2 ml sterile water. Animals were darted with a compressed air dart gun thereby causing the least amount of disturbance possible. They succumbed within 10 - 15 min on either drug combination. Anaesthesia was maintained for 3 h. To facilitate this, another 0,016 mg/kg of M-99 was, on average, administered intramuscularly 50 min and 140 min post initial immobilization. In the last few trials when carfentanil was used with Rompun, only an additional 0,008 mg/kg carfentanil, administered approximately 100 min post initial immobilization, was needed to maintain anaesthesia for 3 h.

Reviving the animals in the case of M-99 induced anaesthesia was accomplished by injecting a morphine antagonist, diprinorphine hydrochloride, or M-50/50 (Reckitt, Durban, S.A.). Approximately double the total M-99 dose was required and two thirds were injected intravenously and the remainder intramuscularly. There was no specific antidote for carfentanil, although M-50/50 was used in the same proportion as for M-99. Under these conditions, the animals revived within 10 min, but were drowsy and required care

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for the following 8 - 10 h. After this period they were more active, but still visibly affected by the drug. Complete recovery was recorded approximately 15 h after the administration of M-50/50.

General methods

Anaesthesia was induced and maintained for the duration of the 3 h required for collecting blood samples. After the animal succumbed to the anaesthetic, the eyes were protected by covering them with towelling. The animal was then weighed and returned to the concrete enclosure where it was placed in the sternal recumbent position. Since the drugs used could have interfered with thermoregulation, the anaesthetized animals were covered with blankets in winter and in summer they were kept cool by spraying with water.

The area surrounding the jugular vein was first cleaned and then swabbed with 70% alcohol. An indwelling jugular catheter (Bard I-Cath, Bard International, U.K.) was inserted and kept in position with surgical tape. A catheter was necessary for the accurate dosage of the tracer substances. For accuracy each substance was contained within its own syringe which enabled weighing before and after injection. The catheter also helped counteract restlessness by reducing the disturbance that would have been caused by the individual injection of each substance.

Initial blood samples, or sample blanks, were collected by drawing

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blood into 10 ml vacuum tubes (Vac-U-Test, Radem Laboratory Equipment, Wynburg, S.A.) containing 14,3 U.S.P. units lithium heparin/ml blood, as anticoagulant. This was followed by the timed injection of the tracer substances. The catheter was then flushed with 40 ml of physiological saline and withdrawn.

Ten to 20 ml blood samples were collected at timed intervals, usually after 5, 10, 20, 30, 45, 60, 90, 120, 135, 150, and 180 min post injection. Samples were either drawn from the femoral veins or the opposite jugular vein from that into which the tracers had been injected.

At the end of the 3 h period, body measurements were recorded, and a long acting penicillin based antibiotic, Compropen (Milborrow & Co., Johannesburg, S.A.) (30000 units/kg body weight) was injected intramuscularly. The animals were then revived.

Analyses of data

Maloiy & Hopcraft (1971) mentioned the usefulness of the body weight^{0,82} exponent in compensating for size differences among genera. In antelope with a low body fat content this exponent becomes particularly useful for comparisons involving water dynamics. However for comparisons involving energy, body weight^{0,75} is more accurate (Kleiber 1961). In the present study, these exponents were used where applicable.

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All results presented are means ± one standard deviation (S.D.) of the mean, for results presented in the text and tables, and means + 1 S.D. of the mean, for those displayed in figures. For statistical comparisons, the Statistical Analysis Systems (SAS Institute Inc, Cary, North Carolina, U.S.A.) General Linear Models Procedure (GLM) was used. Two way analyses of variance were performed on unbalanced data using the Scheffe Multiple Comparison Procedure. This package presents results as either being "significant" or "non-significant" at the desired probability level, and does not provide individual F-values or degrees of freedom. Least squares linear regression analyses and the determination of correlation coefficients were calculated using Statgraphics (Statistical Graphics Corporation, U.S.A.). A significance level of 5% was used to confirm significance in differences of means and variances, unless otherwise stated.

CHAPTER 2

THE EFFECTS OF FRESH WATER, SALINE WATER AND

DEHYDRATION ON SOME BLOOD VARIABLES

INTRODUCTION

In very small organisms, diffusion is sufficient for distribution of respiratory gases. Nearly all large animals, however, have a distribution system based on the movement of a fluid containing not only these respiratory gases, but other constituents (Schmidt-Nielsen 1983). This fluid is the blood, a medium that provides cells with water, oxygen, nutrients and hormones, and removes metabolic waste products (Schalm, Jain & Carroll 1975).

The composition of blood is of utmost importance and ensures that there is little variation in the environment of individual cells throughout the body (Conley 1974). For example, even in the case of disease, plasma Na concentration does not usually change more than 20 mEq/l (Leaf, Couter & Newburgh 1949).

However the complex composition of blood may be altered considerably by stress, starvation, exercise and injury (Russell, Powell, Jones, Winterburn, & Basford 1982), and knowledge of an animals haematology provides an insight into its physiology, and

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its health status (Whittington & Grant 1983).

In the KGNP, gemsbok may be exposed to dehydration, as well as to saline drinking water. Dehydration may cause considerable changes in blood composition, and can lead to death. Maloiy (1972) found that plasma osmolality, and Na, Cl and potassium (K) concentrations all increased during dehydration in the camel (Camelus dromedarius). Increases in plasma Na concentration following dehydration have also been recorded in other species (Donaldson & Edwards 1981; Grey, Naude & Erasmus 1988; Mohamed, Ali & Hassan 1988).

Imbibition of saline water has also been linked to changes in plasma composition. Increases in K and Na concentrations in the plasma of animals given saline water have been reported by Potter (1963), Haines (1964) and Young, Pan & Guyton (1977). The extent of control over such changes and the level of tolerance to them, will influence survival.

In arid regions, periods of dehydration may be followed by rapid rehydration. Tolerance to rapid rehydration is dependent on the rate of efflux of water across the rumen epithelium, the fragility of the red blood cells, and factors which affect this fragility (Turner 1984). Yagil, Sod-Moriah & Meyerstein (1974) reported that the ability of the camel to undergo rapid rehydration was due to its tolerance of extreme changes in red blood cell tonicity.

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However, in the Bedouin goat, rapid rehydration is attributed to slow passage of water from the rumen into the bloodstream, avoiding haemolysis (Wilson 1989).

In vitro testing of the osmotic fragility of the red blood cells of the gemsbok may indicate whether the red blood cells can tolerate osmotic stress. This chapter aims to examine some aspects of the blood physiology of gemsbok under different treatments (See Chapter 1).

MATERIALS AND METHODS

Blood samples were collected soon after the onset of anaesthesia, as described in Chapter 1.

Whole blood analyses

Haematocrit

Haematocrit was determined by drawing whole blood into heparinized micro-capillary tubes and centrifuging at 2500 revolutions per min (rpm) for 20 min at room temperature (25 - 35°C). Haematocrit values were then determined using a plastic overlay with haematocrit scales. No correction was made for plasma trapped in the cell mass.

Haemoglobin

Haemoglobin concentration was measured using the cyanmethaemoglobin method described by Brown (1984). A solution containing sodium bicarbonate, potassium cyanide and potassium ferrocyanide was prepared from a commercial kit (Drabkins reagent, Sigma Chemical Co., U.S.A.). Five millilitres of this solution were pipetted into a test tube and 0,02 ml blood added. The resulting solution was allowed to stand at room temperature for 10 min. During this time potassium ferrocyanide converted haemoglobin from Fe^{2+} to Fe^{3+}, or methaemoglobin. This combined with potassium cyanide to form stable cyanmethaemoglobin (Brown 1984).

The absorbance of the resulting solution was measured using a Bausch & Lomb spectrophotometer set at wavelength 540 nm. Cyanmethaemoglobin reagent was used to set zero absorbance.

A calibration curve was constructed using commercial haemoglobin standards (Boehringer Mannheim, Mannheim, West Germany). The absorbances of the standards were spectrophotomically measured against distilled water at 540 nm, and these values plotted against haemoglobin concentration. A least squares linear regression was calculated, against which all sample values were determined by interpolation.

Mean cell haemoglobin concentration (MCHC) was calculated using the following equation (Ganong 1977) -

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$$\% \text{ MCHC} = \frac{\text{Blood haemoglobin concentration X 100}}{\text{Haematocrit}}$$

Osmotic fragility

Osmotic fragility was determined using the method described by Brown (1984). Buffered NaCl stock solution was prepared by mixing 180 g NaCl, 27,31 g dibasic sodium phosphate, and 4,86 g monobasic sodium phosphate and diluting to 2000 ml. From this stock solution, 200 ml of a 1% (w/v) solution was made by adding 20 ml stock solution to 180 ml distilled water. The pH of the stock solution (pH 7,4) conformed to the described value (Brown 1984).

The following seven concentrations of buffered NaCl solutions were prepared from the 1% solution : 1%, 0,8%, 0,65%, 0,55%, 0,45%, 0,35%, 0,20%. Duplicate aliquots (5 ml) of each solution were placed into individual test tubes, with distilled water being used for the 0% value. Whole blood (0,05 ml) was added to each test tube and gently mixed for one min at room temperature. Each tube was allowed to stand for 30 min at room temperature, after which all tubes were centrifuged (room temperature) at 2000 rpm for 10 min. The spectrophotomic absorbance of the supernatant was determined at wavelength 550 nm in a Bausch & Lomb spectrophotometer. The 1% sample was used to set the zero absorbance value, while the value obtained from the distilled water sample was assumed to represent 100% haemolysis. Percentage fragility was then calculated by

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dividing each value obtained by the 100% haemolysis value.

This test was repeated on three samples of human blood, and on blood from a chemically immobilized female springbok, and a female springbok that had been shot.

Blood volume

Blood volume was calculated by multiplying plasma volume (see . Chapter 4) by (100 - haematocrit value) (Ganong 1977).

Plasma analyses

Plasma was analysed for Na, K, and Cl concentration using an autoanalyzer (Astra, Beckman) at the Institute for Pathology, University of Pretoria. Osmolality of plasma samples was determined by freezing point depression osmometry (u-Osmette, Precision Systems). Plasma protein concentration was determined using a Hitachi portable protein refractometer. Distilled water was used to calibrate this instrument before use.

RESULTS

Haematocrit values were greater in saline acclimated animals than in fresh water acclimated animals in both seasons, with summer values being higher in both treatments than in winter (Fig. 2.). There were no significant differences in haematocrit values between

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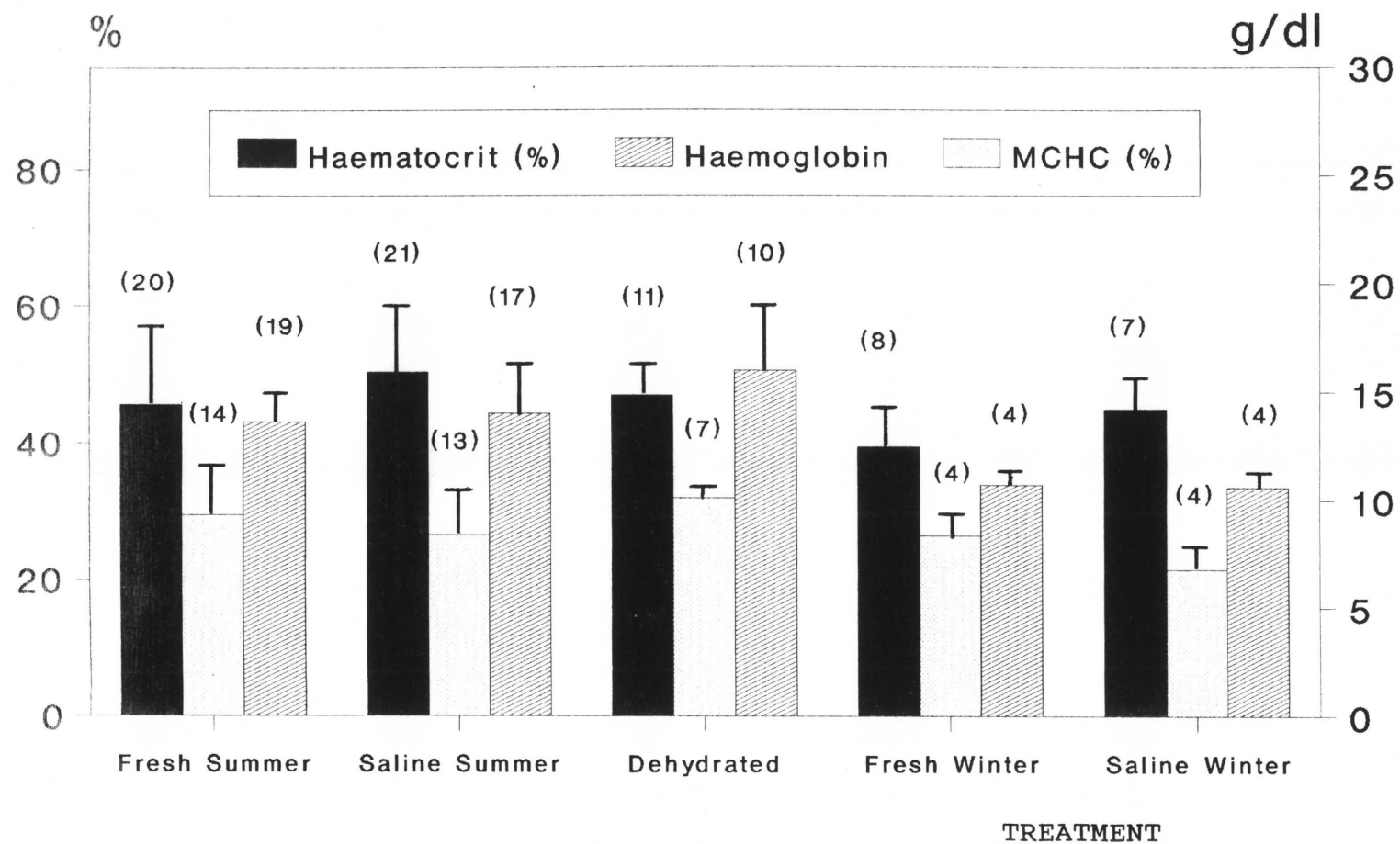
Fig. 2. Haematocrit, haemoglobin and mean cell haemoglobin concentration (MCHC) in gemsbok exposed to different treatments. Results presented as means + 1 S.D. Sample size in brackets.

any treatment.

Haemoglobin concentration, MCHC and haematocrit values for the different treatments are presented in Fig. 2. Haemoglobin concentration was similar within seasons on different water treatments. However, it differed significantly between seasons for similar water treatments. Haemoglobin concentration of animals acclimated to fresh water in summer was also significantly lower than those of dehydrated animals. MCHC was higher in all summer treatments than in winter treatments. Dehydrated animals had the highest MCHC of all treatments. No significant differences were found.

Blood volume scaled to body weight ^{0,82} was larger in summer than in winter. Blood volumes in animals acclimated to fresh and saline water in summer were 0,35 $\pm$ 0,05 1/kg^{0,82} (n = 19) and 0,31 $\pm$ 0,06 1/kg^{0,82} (n = 19), respectively. In dehydrated animals blood volume {0,25 $\pm$ 0,03 1/kg^{0,82} (n = 10)} differed significantly from that of fresh water acclimated animals in summer. Winter values of 0,30 $\pm$ 0,04 1/kg^{0,82} (n = 4) for animals acclimated to saline water and 0,26 $\pm$ 0,03 1/kg^{0,82} (n = 6) for animals acclimated to fresh water, were recorded.

There was a similarity in the shape of the osmotic fragility curves in all treatments (Fig. 3.). Gemsbok blood was also markedly more fragile than human blood in all treatments. The results obtained

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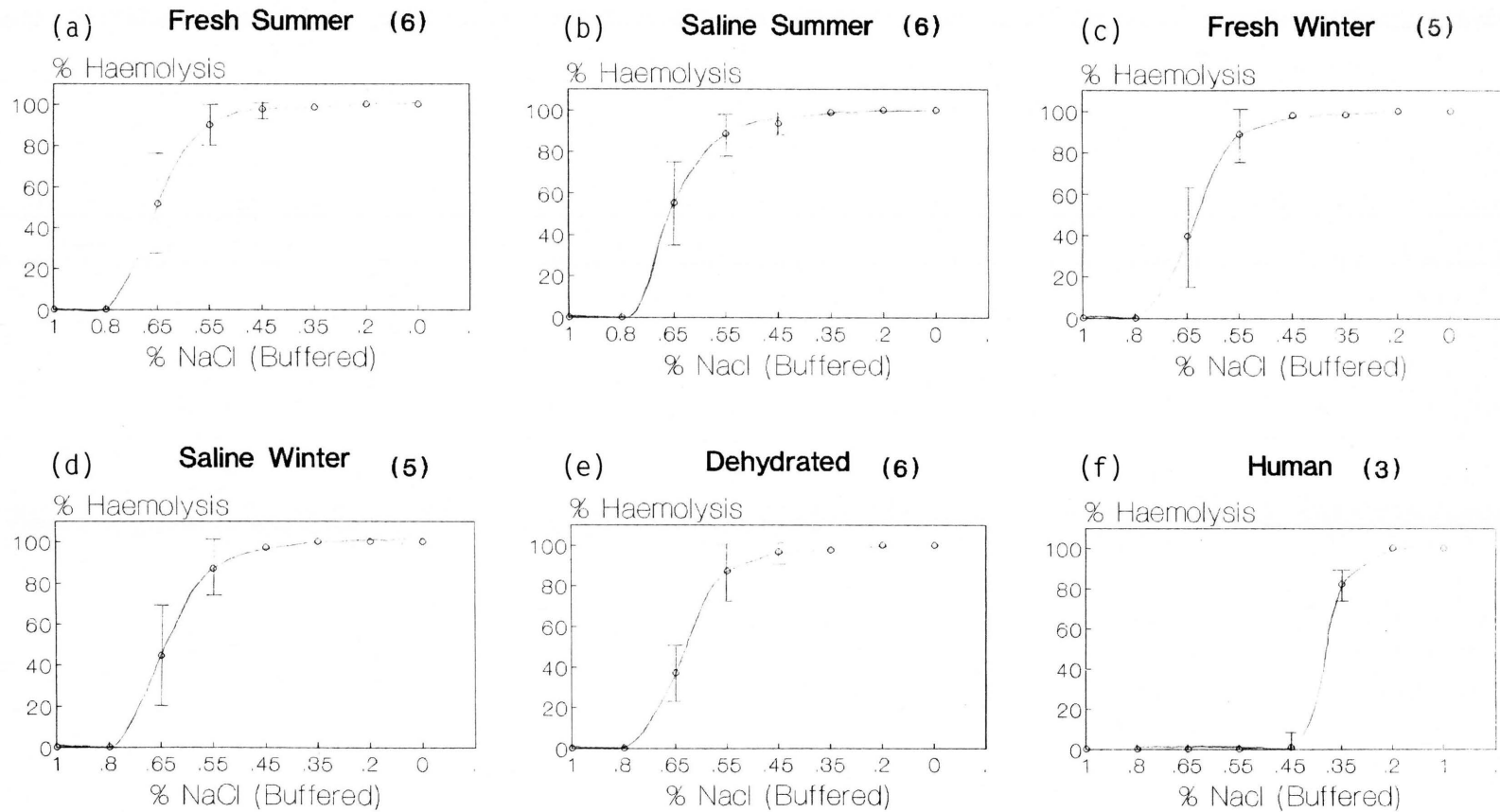
Fig. 3 (a-e). Osmotic fragility curves for gemsbok exposed to different treatments.(f) Human red blood cell osmotic fragility curve for reference. Results presented as means (o) ± 1 S.D. (vertical bars). Sample size in brackets.

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for human blood are of the same order as those published (i.e. Brown 1984). Blood samples from the shot and chemically immobilized springbok were similar in fragility (Table 2.), and were also similar in fragility to gemsbok blood.

In summer, plasma osmolalities of fresh and saline acclimated animals were $314,8 \pm 16,69$ mOsm/kg H_2O (n = 22) and $321,0 \pm 19,21$ mOsm/kg H_2O (n = 23), respectively. Plasma osmolality was lower in winter with values of $301,5 \pm 14,88$ mOsm/kg H_2O (n = 8) and $299,0 \pm 6,60$ mOsm/kg H_2O (n = 7) recorded in fresh and saline acclimated animals, respectively. Dehydrated animals had the highest plasma osmolalities with a mean of $338,0 \pm 17,26$ mOsm/kg H_2O (n = 14). This was significantly different from results obtained in animals acclimated to fresh water in summer.

Plasma Na concentrations in fresh and in saline water acclimated animals in summer were higher than those recorded in winter (Fig. 4.), although not statistically significant. Dehydrated animals had the highest mean value (175,3 mmole/l), a value which differed significantly from fresh water acclimated animals in summer (161,1 mmole/l), but not from that of saline acclimated animals in summer (168 mmole/l). Plasma Na concentration correlated significantly with plasma osmolality ($r^2=0,92$; p<0,001) for all treatments, as did plasma Cl concentration ($r^2=0,81$; p<0,001). In addition the concentration of plasma Cl closely followed that of Na ($r^2=0,83$; p<0,001). Plasma Cl concentration was higher in summer than in

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Table 2. Comparison of red cell osmotic fragility in shot and chemically immobilized female springbok, _Antidorcas marsupialis_. Sample size in brackets.

% NaCl (Buffered)	% Haemolysis	
	Shot (n=1)	Chemically immobilised (n=1)
1	0	0
0,85	4	2
0,65	20	22
0,55	81	76
0,45	100	96
0,35	100	100
0,20	100	100
0	100	100

24a
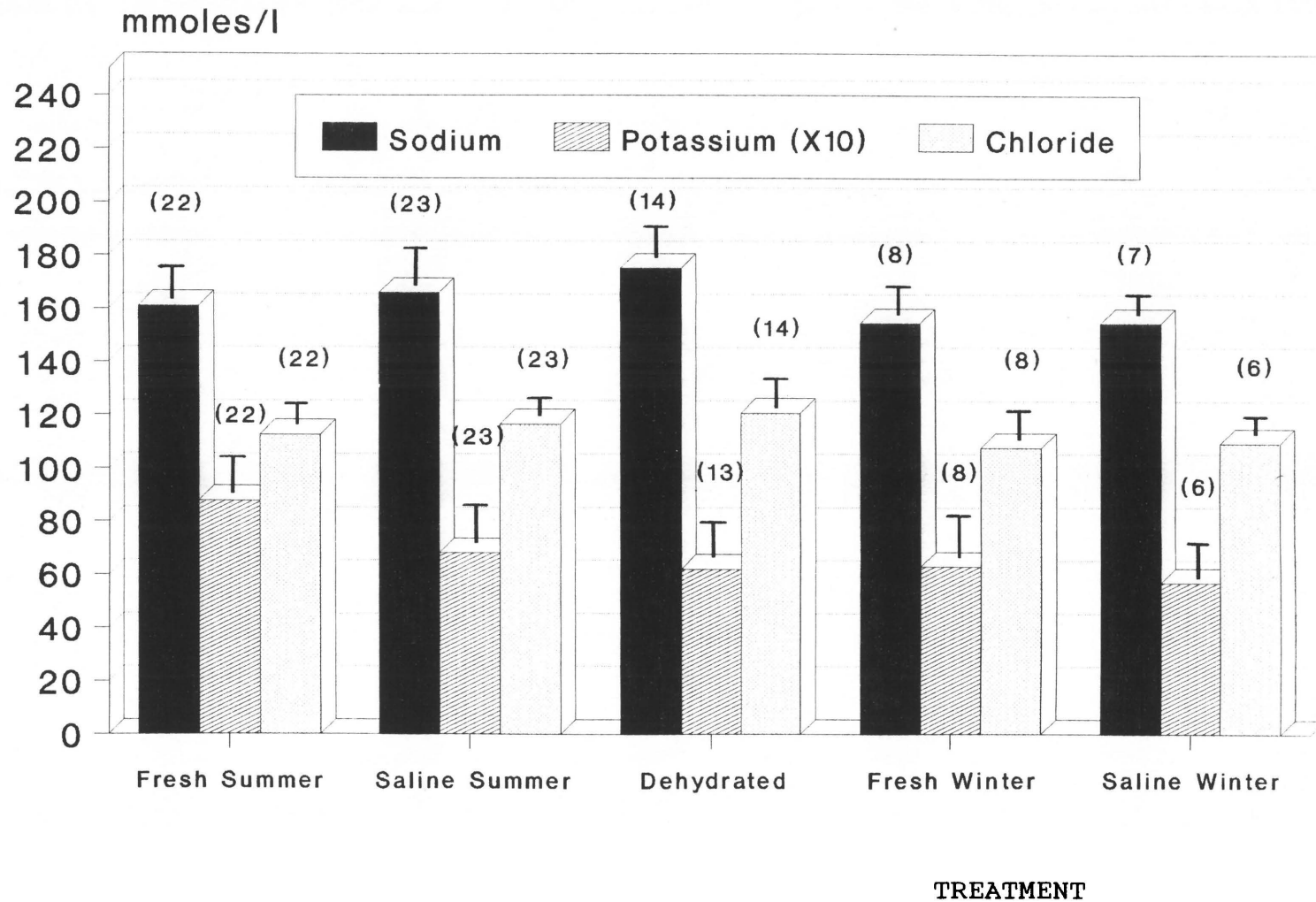
Fig. 4. Plasma electrolyte concentrations in gemsbok exposed to different treatments. Results presented as means + 1 S.D. Sample size in brackets.

winter. Plasma Cl concentration in dehydrated gemsbok differed significantly from that recorded in fresh water acclimated animals in summer.

Plasma K concentration was not significantly affected by water treatment. However, plasma K concentrations were higher in hydrated animals in summer than in winter.

In summer, plasma protein concentration was $6,7 \pm 0,6$ g/l (n = 22) in fresh water acclimated animals and $6,8 \pm 0,6$ g/l (n = 23) in saline acclimated animals. In winter, values were lower with $6,4 \pm 0,7$ g/l (n = 8) and $6,3 \pm 0,5$ g/l (n = 7) being recorded in fresh water and saline water acclimated animals, respectively. None of these values differed significantly. Plasma protein concentration was higher in dehydrated animals ($7,3 \pm 0,4$ g/l; n = 14) than in hydrated animals, and was significantly different from saline water acclimated animals in winter.

DISCUSSION AND CONCLUSIONS

Whole blood

Haematocrit values recorded in the present study were similar to those found by Pospisil, Kase, Vahala & Mouchova (1984) in gemsbok. Pospisil et al. (1984) also found that the haemoglobin concentration in captive gemsbok was slightly greater than 12 g/dl,

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not only comparable with the mean value for this study, but also much lower than that for most other antelope species investigated by them.

Initially it was believed that this may have been due to the effects of the immobilizing drugs, since Drevemo & Karstad (1974, in Pospisil *et al.* 1984) recorded decreased haemoglobin and haematocrit levels in impala, _Aepyceros melampus_, after administration of xylazine and/or etorphine hydrochloride, as did Fourie & Van Ouwerkerk (1984) in gemsbok. However the markedly similar within-season haemoglobin values, and the differences between seasons found in the present study imply that other factors may also be responsible.

Within seasons, the blood haemoglobin concentration and haematocrit remained fairly constant, although lower in winter than in summer. This was also reported for Grevy's zebra (_Equus grevyi_), (Pospisil, Kase & Vahala 1989). At present no explanation for this observation can be given.

In summer, blood volume was only significantly reduced in dehydrated animals compared with blood volume in gemsbok acclimated to fresh water. Since the haematocrit level was similar to that of animals acclimated to fresh water in summer, it is likely that this resulted from both a loss of plasma and a reduction in red blood cell volume. The reduction in cell size would result in a similar

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haematocrit (to hydrated animals) being recorded even if haemoconcentration was occurring. Evidence for the decrease in the size of red blood cells is indirect. Firstly, plasma protein concentration, recognized as a more reliable indicator of hydration status than haematocrit (Carlson, Rumbaugh & Harrold 1979), and plasma osmolality, were both higher in dehydrated animals than in hydrated animals. This indicates that dehydration has occurred, with the result that osmotic forces may have removed fluid from the red cell. Secondly, the increase in MCHC indicates a reduction in red cell volume without a corresponding decrease in the haemoglobin content of the cell (Lawry 1977).

One possible advantage of this would be to minimize an increase in blood viscosity as plasma volume declined, thereby facilitating blood flow in the dehydrated animal. Since red blood cell volume is believed to have declined, any haemoconcentration that might be occurring, based on haematocrit evaluation, would be less detectable. Haematocrit, under these conditions, therefore loses its value as an indicator of hydration status.

The results of the osmotic fragility tests showed that gemsbok red blood cells were more fragile than human red blood cells. There are many reports in the literature of decreased fragility of the red blood cells (in vitro) of arid dwelling species (Perk 1963; Hawkey 1975; Altman & Dittmer 1961, in White & Brown 1981). Chosniak & Shkolnik (1977) however, found that the red blood cells of the

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Bedouin goat, <u>Capria</u> <u>hircus</u> (regarded as arid adapted), were also relatively fragile.

It was at first thought that the test was providing inaccurate results. Yet the haemolysis of human red blood cells in exactly the same solutions followed the sigmoid curve for normal human red blood cells (Brown 1984). The effects of the drugs used in anaesthesia were also suspected as a possible cause of error since Hughes-Jones (1973) found that drugs can interfere with lipid components of the red blood cell. However blood obtained from both chemically immobilized and shot (culled with a single bullet) springbok, <u>Antidorcas</u> <u>marsupialis</u>, had similar osmotic fragilities, which resembled that of the gemsbok (Table 2 & Fig. 3.). Therefore it can be assumed that the anaesthetic played little, if any, part in influencing the fragilities.

It was initially believed that the blood of the gemsbok would be resistant to haemolysis because the animal is exposed to periods of water restriction, followed by access to water. In cattle, rapid rehydration following dehydration led to haemolysis (Bianca 1970; Maloiy & Boarer 1971). However in some arid dwelling ruminants subjected to periods of water deprivation, large quantities of water can be drunk following dehydration without ill effect. Coupled to this are reports that changes occur slowly in fluid levels (Chosniak & Shkolnik 1977).

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Since the in vitro tests showed that the red blood cells of the gemsbok are extremely fragile, it may be concluded that should the circulatory system be stressed by similar osmotic forces, large scale haemolysis would result. It is however recognized that in vitro osmotic fragility tests attempt only to correlate a possible similar behaviour of the cells in vivo. The actual relationship between in vivo and in vitro responses is unknown. It is believed that in vivo, there are many factors that would counter the effects of an osmotic perturbation i.e. electrolyte movements (Wintrobe 1961). Although the cells were more fragile in gemsbok than in humans, there must still be a large influx of fresh water into the system before haemolysis of gemsbok red blood cells even begins. Such an influx would have to be severe enough to reduce plasma osmolality to around 200 - 220 mOsm/l. Since it is highly unlikely that large scale haemolysis occurs after the ingestion of large quantities of water, it appears that there may be either a gradual release of water from the gastrointestinal tract into the circulatory fluid, as found by Chosniak & Shkolnik (1977), or that the response to an osmotic stress (ie. influx of water) is rapid and efficient. It is not known why the red blood cells of the gemsbok are extremely fragile in vitro, and further research is required.

Plasma electrolytes

Macfarlane, Morris, Howard, McDonald & Budtz-Olsen (1961) found that plasma Na concentration increased in sheep, <u>Ovis aries</u>, by 5%

to 152 - 156 mEq/l following four days of dehydration. After five days the increase was 10%. Dehydrated gemsbok showed an 8,8% increase in plasma Na concentration over the same period, compared with fresh water acclimated animals in summer.

While increases in plasma Cl concentration occurred in dehydrated animals, the increase (6,2% from fresh water acclimated animals in summer) was lower than in the Dorcas gazelle, Dorcas gazella, where plasma Cl concentration increased by approximately 8% after five days of dehydration (18% when dehydrated for 10 days) (Mohamed et al. 1988).

Dehydration induced changes in plasma K concentration are more varied. Following four days of dehydration in sheep, Macfarlane et al. (1961) reported that plasma K concentration decreased by 3%. After five days the change was 1%. Grey et al. (1988) also recorded decreased plasma K concentration after dehydration in the ostrich, Struthio camelus, yet Senay & Christensen (1965) found no significant differences between the plasma K concentration during hydration and dehydration. McKinley, Denton, Nelson & Weisinger (1983) found that plasma K concentration either remained constant or declined. Rugangazi & Maloiy (1988) found increased plasma K concentration in dik-dik, Rhynchotragus kirkii, following dehydration, as did Mohamed et al. (1988) who recorded a 44% increase in the plasma K concentration following 10 days of dehydration in the Dorcas gazelle.

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Plasma K concentration was lower in dehydrated gemsbok than in hydrated animals in summer. Thrasher, Wade, Keil & Ramsay (1984) noted in their study that plasma K concentration decreased during dehydration in the dog, <u>Canis</u> <u>familiaris</u>. This they attributed to a reduced intake of K and not to increased excretion. Although dehydrated gemsbok reduced food intake, it is unlikely that the level of K intake would fall to such low levels as to affect plasma concentration, especially since lucerne (the diet on which they were maintained) is likely to contain large amounts of K (Crampton & Harris 1969).

A more likely explanation is based on proposed changes in renin and aldosterone levels. The decrease in the extracellular fluid volume (ECFV) would most likely cause a decrease in mean arterial pressure, which in turn would stimulate increased renin and aldosterone secretion (Ganong 1977). The effect of this would be to increase Na reabsorption in the collecting duct of the kidney, while increasing K excretion (Ganong 1977). As a result plasma K concentration declines while plasma Na concentration increases.

Dehydration resulted in a significant increase in plasma osmolality in gemsbok. This has also been noted in many other species (Haines 1964; Dunn, Brennan, Nelson & Robertson 1973; Donaldson & Edwards 1981; Grey <u>et</u> <u>al.</u> 1988; Rugangazi & Maloiy 1988). Plasma osmolality increased to 460 mOsm/kg H_2O in a camel dehydrated for 45 days (Maloiy 1972), while Maloiy & Boarer (1971) reported plasma

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osmolalities of 360 mOsm/kg H_2O in dehydrated donkeys, _Equus sinus_.

Given saline drinking water, Donaldson & Edwards (1981) found no difference in plasma osmolality, Na or Cl concentrations after five days in the gerbil, _Meriones ungiuculatus_. Maloiy (1972) found that plasma osmolality was either unchanged in the camel, or changed only slightly in the donkey, when given saline water. In the present study, changes were noted between animals acclimated to fresh and saline water in summer although these were not significant. In winter, differences between treatments were also insignificant. This is indicative of the ability to control plasma electrolyte concentrations when given saline drinking water.

Seasonality also plays an important part in determining plasma electrolyte concentrations. Seasonal changes in plasma electrolyte concentrations can be expected as a result of changes in ambient temperature and the response by gemsbok through their water metabolism. It will be argued in subsequent chapters that winter is less stressful than summer with respect to water metabolism. This is based on the observation that in winter, gemsbok had lower osmolalities, Na, K and Cl concentrations than in summer on both fresh and saline water. This is similar to the finding by Weeth & Haveland (1961, in Wilson 1966b) that in sheep in summer, serum Na and K concentrations were increased when drinking water contained 1,2% NaCl, but not in winter when given 1,5% NaCl.

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To conclude, it is apparent that gemsbok are able to regulate the blood variables measured in this study over a wide range of external conditions. There is little change in plasma electrolyte concentrations when exposed to saline water. When dehydrated, plasma protein concentration increased, and is believed, through the effects of an increased colloid osmotic pressure, to contribute to a retention of water in this compartment during dehydration.

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CHAPTER 3

ASPECTS OF RENAL RESPONSE TO FRESH WATER,

SALINE WATER AND DEHYDRATION

INTRODUCTION

Within the body, fluid composition and volume would fluctuate markedly were it not for the stabilizing effects of homeostatic mechanisms. In arid environments where water supplies may be limiting, the animal's ability to regulate body fluid composition is particularly crucial, and one of the most important mechanisms for controlling water and electrolyte balance in the body is the adjustment of renal function (Berlyne 1980; Ganong 1981). The kidney's role is, in part, to defend the volume and tonicity of the body fluids since it is of fundamental importance to the continued existence of the organism. For example, K regulation must be very closely controlled since it plays a role in the function of nerve and muscle tissue. Serious imbalances in this electrolyte can lead to death (Vander 1975).

The extracellular fluid is the immediate fluid regulated by the kidney, and one of the most important generalizations in body fluid physiology is that the volume of the extracellular fluid depends largely on the Na content of that fluid, and on total body Na

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(Vander 1975). Electrolyte balance is thus a fundamental part of renal function.

The renal function of arid dwelling species must be flexible enough to counter the unpredictable environmental conditions. Yet, as with many other physiological variables, some species are able to tolerate greater changes than others. Macfarlane et al. (1961) found in their studies that in adapting to water deprivation, the kidney not only played one of the primary roles, but that towards the end of dehydration, kidney function was the most important determinant of plasma composition.

The extent to which some renal variables are altered by the experimental treatments, is the subject of the present chapter.

MATERIALS AND METHODS

Introduction

Renal function is a complex interaction of a number of processes, the most important being glomerular filtration rate (GFR), renal blood flow (RBF) and tubular absorption and secretion. Each of these has an effect on the regulation of body fluid volumes and osmolalities in its own right.

Measurements of GFR and the effective renal plasma flow (ERPF) are

readily carried out using clearance techniques. These are essentially measures of input-output relationships of either endogenous or exogenous substances, where excretion of the substance is, ideally, wholly via the kidney. Furthermore, they are valuable in appraising the effects of systemic factors or substances on renal function (Maack 1986).

The single injection technique circumvents many of the shortcomings associated with the "classical" constant infusion technique. These include the need for a constant plasma concentration of the tracer substance, timed urine collection, and determination of constant urine flow rates (Mandel, Vidt & Sapirstein 1955). All of these would be difficult to perform on gemsbok, since the insertion of a bladder catheter is problematic due to an S-shaped urethral process in the male (pers. obs.). Furthermore, constant infusion into an animal which is liable to move was deemed impossible (as periods of kicking, attempting to stand up and thrashing of horns were common even during anaesthesia).

Using the single injection technique, the disappearance of a suitable tracer substance from the blood is studied following the administration of a single bolus (known amount) of the substance. Scheer & zum Winkel (1971) defined clearance in this case to be "that volume of plasma in millilitres cleared of the test substance per minute".

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Theoretically any substance used for GFR determinations must be freely filterable at the glomerular / Bowmans capsule membrane. It must be neither synthesized nor destroyed in the tubules; it must be chemically stable and detectable. Furthermore, it must neither be absorbed nor secreted in the tubules (Smith 1951). Moreover, Levinsky & Levy (1973) defined the limits of substances used for clearance studies by stating that such substances should not be protein bound, as filtration will not occur. The assumptions behind the use of such a perfect substance are that it is instantaneously distributed in a constant volume and that it should have a constant disappearance over a wide range of plasma concentrations (Levinsky & Levy 1973).

For ERPF determinations the substance must be freely filterable and it must be secreted but not reabsorbed by the renal tubular cells. In addition, a single passage of blood through the kidney should result in complete clearance of the tracer from the blood. In other words, the theoretical extraction ratio should be 1.

As with many techniques, theory and practicality may not be intimately related, and problems in finding a substance which meets the criteria set above have been encountered.

Many suitable substances have been suggested. In GFR & ERPF determinations, inulin and para-aminohippuric acid (PAH) respectively, have been used, since both substances fulfil most of

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the requirements mentioned above. However they lacked accuracy. The large molecular size of inulin retarded its diffusion into the body compartments (Levinsky & Levy 1973). It was also barely soluble and required timed and complete urine collections.

Mandell et al. (1955) found evidence for intracellular penetration of PAH and, as such, the volume of distribution exceeded the ECFV. Furthermore, and more importantly, PAH was cleared so rapidly from the system that it introduced error. Mandell et al. (1955) found this error to be 10 - 25% and 25 - 50% in different periods, and suggested that the faster a substance is cleared, the greater the error occurring in the mixing period.

Since no substance mixes instantaneously, a mixing period is always evident, and is followed by an excretion period which is essential in the determination of the disappearance of the tracer from the blood. Mandell et al. (1955) indicated that if the mixing period is not evaluated, the plasma disappearance curve dominated by the mixing process will be confounded by the shortage of information regarding excess excretion during the mixing period. Harris & Gill (1981) agree that the rate of entry into the tubule is much greater in the initial period, and if GFR remains constant, a percentage of the substance will be filtered in the time to equilibration. However, unless GFR is increasing dramatically, in which case an underestimate will arise (and vice versa), the resulting error will be small.

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In the case of PAH, Newman (1949, in Mandell et al. 1955) found that the clearance of PAH was not independent of plasma PAH concentration as plasma PAH concentration declined. The accuracy of PAH in renal plasma flow determinations, is therefore questionable.

More recently, the use of isotopically labelled substances has received a great deal of attention. The advantage in using an isotope is the ease and accuracy with which it can be assayed. Of the substances tested, ethylenediaminetetraacetic acid (EDTA) labelled with Chromium-51 (Cr^{51}) was found suitable for GFR determinations by Myers & Dienar (1960). Forland, Pullman, Lavender & Aho (1966) and Favre & Wing (1968) found good correlations between inulin clearance and Cr^{51} EDTA clearance. It is not certain whether Cr^{51} EDTA is protein bound or not. Knapp & Walker (1967, in Heath, Knapp & Walker 1968) and Garnett, Parsons & Veall (1967) found no evidence for this, although Stacy & Thorburn (1966) suggested from indirect evidence that 2% of Cr^{51} EDTA may become protein bound. In addition, Heath et al. (1968) found that it underestimated GFR relative to inulin clearance. They believed this was due to Cr^{51} EDTA being handled differently in the kidney since it was partially protein bound and, in addition, there may have been some tubular reabsorption.

Controversy also surrounds the question as to whether or not Cr^{51} EDTA is reabsorbed or secreted in the tubules. Favre & Wing (1968) and Forland et al. (1966) found no evidence for this. However

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Forman & Trujillo (1954, in Heath et al. 1968) used C^{14} EDTA and found evidence for tubular secretion.

To summarize, despite its possible limitations in fulfilling the requirements for GFR determinations, Cr^{51} EDTA has been the preferred tracer in GFR determinations for many years. Groth & Aasted (1984) report that for the last ten years it has been used as a standard method of evaluation in children. Furthermore, Levinsky & Levy (1973) report that it seems reliable and Vogeli, Riedwyl, Donath & Oetliker (1971) found a correlation coefficient of 0,991 against inulin and concluded it was reliable. They also found that the radiation dose was minimal with a half life value of only 60 min in the liver.

Iodine-131(I^{131}) or Iodine-125 (I^{125}) labelled orthoiodohippuric acid (OIA) were deemed accurate for ERPF determinations (Tubis, Posnick & Nordyke 1960). Containing one atom of iodine, it has the highest renal uptake of all iodine compounds. It is almost exclusively secreted by the tubular cells, and it has a lower radiation dose than I^{131} making it safer to use (Scheer & zum Winkel 1971). Briggs & Boyle (1965) found that it correlated well with PAH and that approximately 83% of this substance was cleared from the bloodstream in a single passage through the kidney. Heinz, Hor, Steinhoff & Hadid (1965, in Scheer & zum Winkel 1971) reported that the problem with using iodinated compounds was that deiodination may occur, and that this could be as high as 2 - 15% with I^{131}.

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After renal filtration, free iodine may be absorbed and thus false results could be obtained.

Briggs & Boyle (1965) and Hesse, Uthgenannt & Ludwig (1967, in Scheer & zum Winkel 1971) found good agreement between single injection and constant infusion techniques. This was supported by Vogeli _et al._ (1971) who found that I^{125} OIA correlated to 1,007 with PAH, and like Cr^{51} EDTA it also had a short half life of 30 min in the liver.

Experimental application

Based on the aforementioned assessment, Cr^{51} EDTA and I^{125} OIA were chosen to measure GFR and ERPF respectively. The experimental protocol in Chapter 1 describes the procedure that was followed.

i) GFR and ERPF determination.

A blood sample (10 ml) was taken from each animal before 120 uCi Cr^{51} EDTA and 35 uCi I^{125} OIA (Amersham International, Amersham, U.K.) were injected via the indwelling jugular catheter. Syringes were weighed before and after injection to determine the mass of injected substance. Standards were retained for the determination of radioactivity and subsequent calculation of injected radioactivity.

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Blood samples were withdrawn at intervals as described in Chapter 1. Plasma, collected after centrifuging blood samples (3000 rpm; 20 min; room temperature), was immediately frozen and stored until analysed.

Analyses were carried out in the Department of Nuclear Medicine, Medical Faculty, University of the Witwatersrand. Plasma samples (1 ml) were placed in counting vials and counted for 10 min (20 min if time allowed) on a dual channel gamma counter (5320 Auto Gamma, Packard Instruments, Chicago, U.S.A.). One channel was set to count Cr^{51} at 320 KeV and the other at 27 KeV to count I^{125}. Standards were diluted and counted in the same manner, and background radiation was corrected for.

Results for both GFR and ERPF were mathematically analysed using a bi-exponential equation. This is represented graphically in Fig. 5. Initially there is a rapid decline in plasma radioactivity (X) (rapid exponential) until equilibrium with the extracellular fluid has been attained (E). Thereafter the decline in radioactivity is linear (slow exponential), which represents renal excretion. Following regression analysis on this linear region, a straight line is generated to cut the y-ordinate (B). Point by point subtraction of the linear extrapolation from the original curve gives Y, which cuts the Y-ordinate at A.

Mathematically this is analysed using the following equation (Cr^{51}

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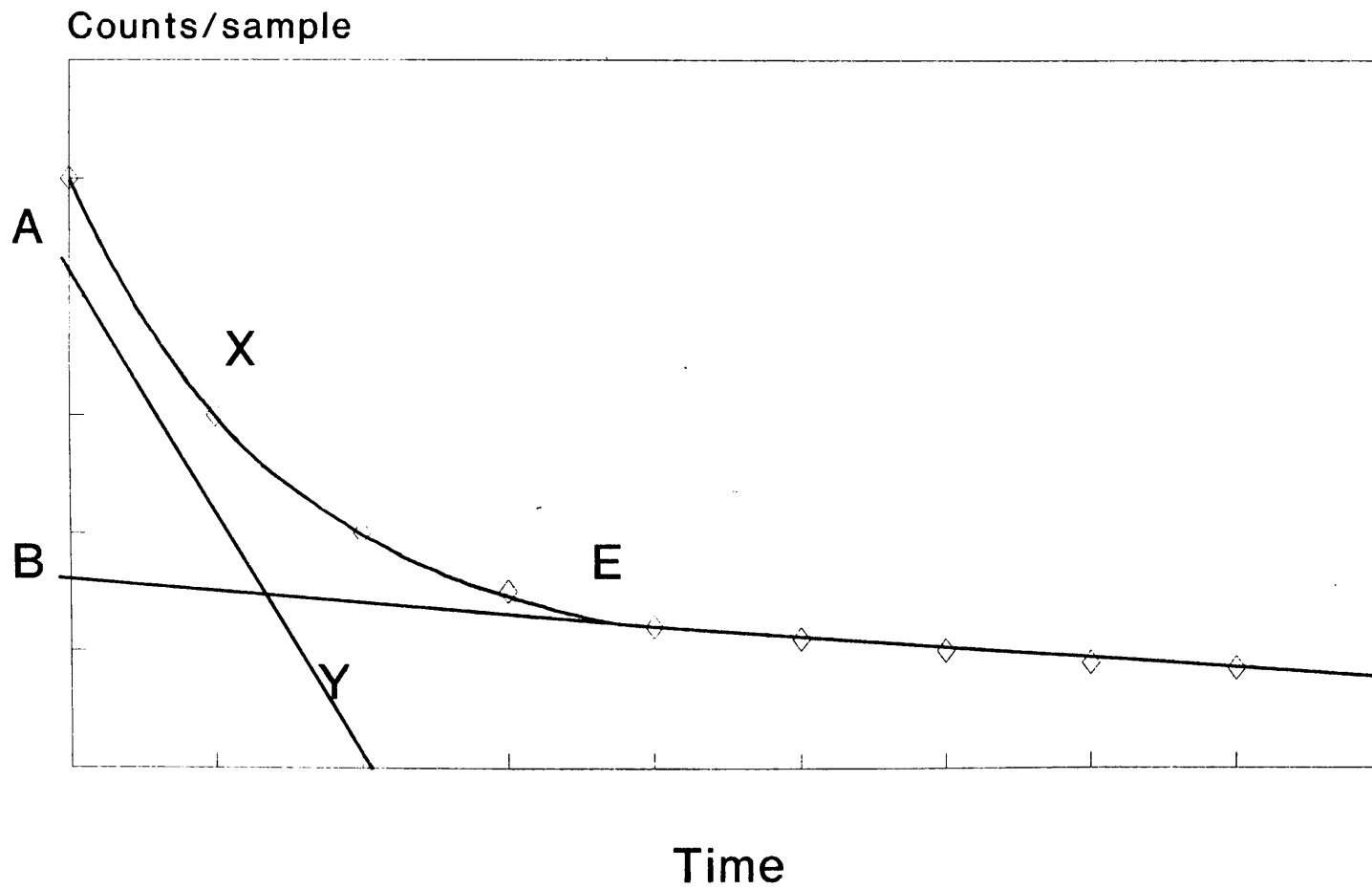
Fig. 5. Resolution of tracer decay curve into
bi-exponential components (see text for explanation of
symbols).

EDTA pack leaflet, Amersham International, Amersham, U.K.) -

$$\text{GFR or ERPF} = \frac{I \ Y_1 \ Y_2}{AY_2 + BY_1}$$

Where - I = injected radioactivity less
        background count

       A = logarithm of the intercept
        of rapid exponential

       B = logarithm of the intercept
        of slow exponential

       Y_1 = slope of rapid exponential

       Y_2 = slope of slow exponential

Effective renal blood flow (ERBF) was calculated following
Gottschalk & Lassiter (1974):

$$\text{ERBF} = \frac{\text{ERPF}}{(1 - \text{haematocrit})}$$

The filtration fraction (FF) was calculated following Harvey (1974)

$$\text{FF} = \frac{\text{GFR}}{\text{ERPF}}$$

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The filtered load of a substance, Y, was calculated following Yagil & Berlyne (1976) :

$$\text{Filtered load} = \text{GFR} \times \frac{\text{plasma concentration of Y}}{1,00}$$

To account for metabolic differences in the animals due to size, GFR, ERPF and RBF are expressed as body weight ^{0,75} as suggested by Edwards (1975).

Urine variables

Urine Na, K and Cl concentrations were determined in an autoanalyzer (Astra, Beckman) at the Institute for Pathology, University of Pretoria. Prior to analyses, urine samples were diluted 1:10 in distilled water. Osmolality was determined by the use of a freezing point depression osmometer (u-Osmette, Precision Systems).

The urine osmolality / plasma osmolality ratio (U/P ratio), an indication of kidney concentrating ability (Gordon 1977), was calculated by dividing urine osmolality by plasma osmolality.

RESULTS

GFR and ERPF values are shown in Table 3. In summer, the mean GFR value for dehydrated animals differed significantly ($p < 0,1$) from that of animals acclimated to fresh water. The lower value for

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Table 3. Glomerular Filtration Rate (GFR), Effective Renal Plasma
Flow (ERPF) and Filtration Fraction in gemsbok exposed to
different treatments. Results presented as means $\pm$ 1 S.D.
Sample size in brackets.

PARAMETER / TREATMENT	GFR (ml/min/kg$^{0.75}$)	ERPF (ml/min/kg$^{0.75}$)	FILTRATION FRACTION
Fresh Summer	7,49 $\pm$ 2,2 (15)	47,35 $\pm$ 20,5 (12)	0,18 $\pm$ 0,07
Saline Summer	6,69 $\pm$ 2,2 (15)	40,76 $\pm$ 12,5 (10)	0,17 $\pm$ 0,07
Dehydrated	5,11 $\pm$ 2,7 (14)	24,16 $\pm$ 7,6 (10)	0,21 $\pm$ 0,05
Fresh Winter	6,70 $\pm$ 1,5 (5)	57,21 $\pm$ 5,4 (8)	0,12 $\pm$ 0,04
Saline Winter	7,44 $\pm$ 2,7 (4)	41,21 $\pm$ 5,4 (5)	0,16 $\pm$ 0,07

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saline acclimated animals did not differ significantly from that of fresh water acclimated animals. In winter, GFR was higher in saline water acclimated than in fresh water acclimated animals, although non-significantly. Comparing winter and summer values, GFR was lower in fresh water acclimated animals during winter than in fresh water acclimated animals in summer. With saline treatments, GFR in winter exceeded that in summer, although non-significantly.

ERPF was significantly different between dehydrated animals and animals acclimated to fresh water in summer and winter.

ERBF in summer was $97,0 \pm 33,6$ ml/min/kg^{0,75} (n = 12) in fresh water acclimated animals, and $96,0 \pm 34,0$ ml/min/kg^{0,75} (n = 10) in those acclimated to saline water. In winter, values recorded were $95,0 \pm 21,4$ ml/min/kg^{0,75} (n = 6) and $80,0 \pm 15,0$ ml/min/kg^{0,75} (n = 5) for fresh and saline water acclimated animals, respectively. In dehydrated animals ERBF was significantly lower than in fresh water acclimated animals in summer, with a value of $38,2 \pm 5,5$ ml/min/kg^{0,75} (n = 10). Little difference in ERBF was apparent in hydrated animals irrespective of treatment or season, but when dehydrated, a significantly different result was obtained.

Filtration fraction (the ratio of GFR to ERPF) tended to be higher in summer than in winter, and was highest in dehydrated animals (Table 3.). The differences were however not significant.

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The filtered loads of Na, K and Cl are shown in Table 4. Although no significant differences between any treatments were found, the lowest filtered loads of these electrolytes occurred in dehydrated animals.

The concentration of urine electrolytes measured is shown in Fig. 6. Dehydrated animals had a significantly lower urine Na concentration than animals acclimated to saline drinking water in summer and winter. Significantly higher urine Na concentrations, relative to fresh water acclimated animals, were also found in animals acclimated to saline water in both summer and winter. Urine K concentration was higher in animals acclimated to saline water than in animals acclimated to fresh water in both summer and winter. Dehydrated animals had the highest urine K concentration, although none of these values differed significantly.

Urinary Cl concentrations were higher in nearly all hydrated treatments than corresponding Na values. Chloride concentrations were greater in saline acclimated animals than in animals acclimated to fresh water in both summer and winter. Dehydrated animals had a lower Cl concentration than Na concentration in their urine, and this was significantly different from animals acclimated to saline water in both seasons, and fresh water in winter.

The osmolality of the urine and plasma is shown in Fig. 7. A significant difference in urine osmolality was found between

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Table 4. Filtered load of electrolytes in gemsbok exposed
to different treatments. Results presented as means
± 1 S.D. Sample size in brackets.

Electrolyte / Treatment	Sodium	Potassium (mmole / min)	Chloride
Fresh Summer (14)	49,8 ± 13,6	1,8 ± 0,6	33,1 ± 10,4
Saline Summer (13)	46,9 ± 17,7	1,8 ± 0,8	32,8 ± 12,6
Dehydrated (14)	32,5 ± 11,0	1,1 ± 0,7	22,4 ± 5,5
Fresh Winter (5)	38,4 ± 8,3	1,5 ± 0,3	26,9 ± 6,0
Saline Winter (4)	48,9 ± 16,9	1,7 ± 0,5	34,7 ± 12,3

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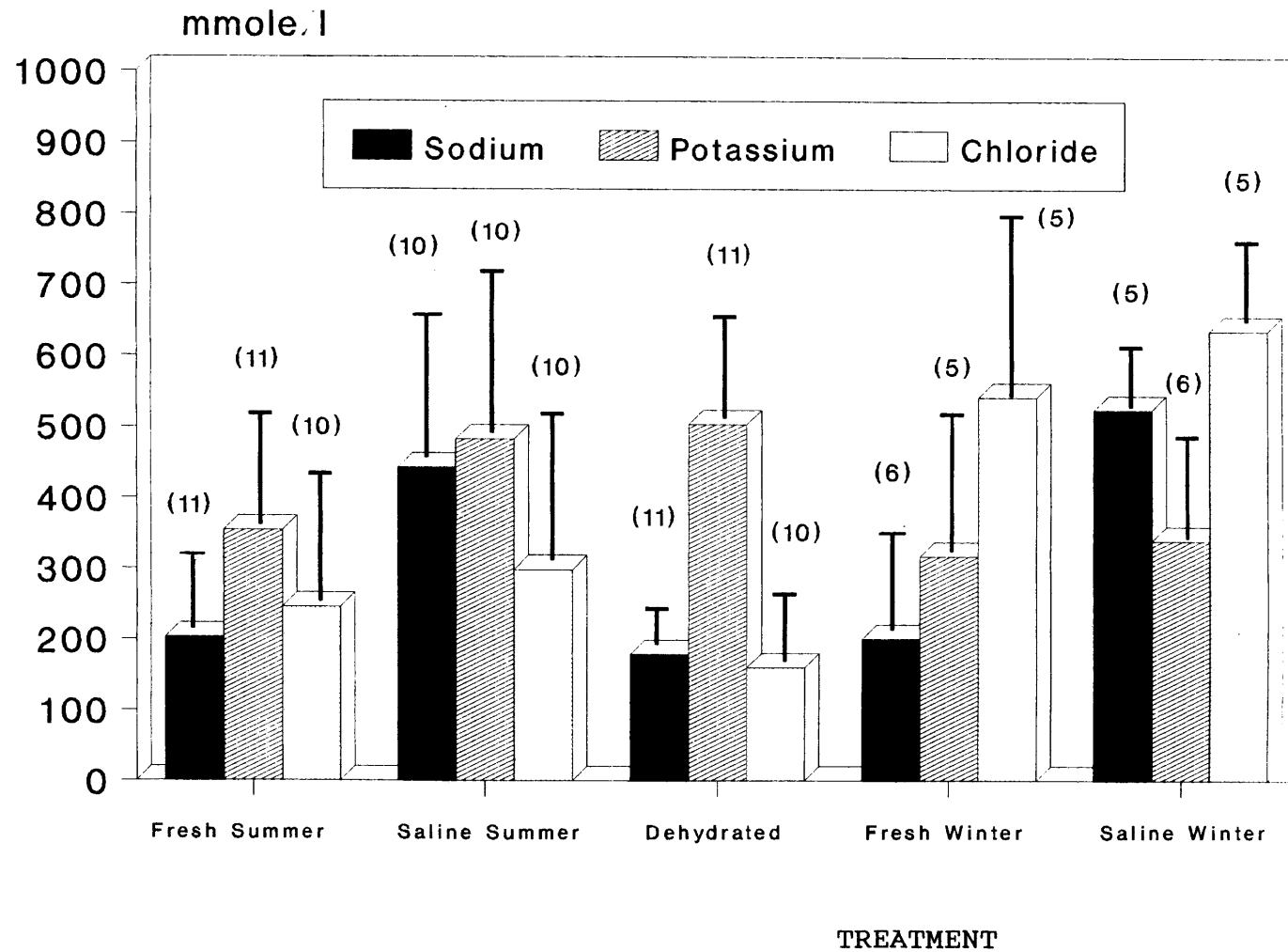
Fig. 6. Urine electrolyte concentrations in gemsbok exposed to different treatments. Results presented as means + 1 S.D. Sample size in brackets.
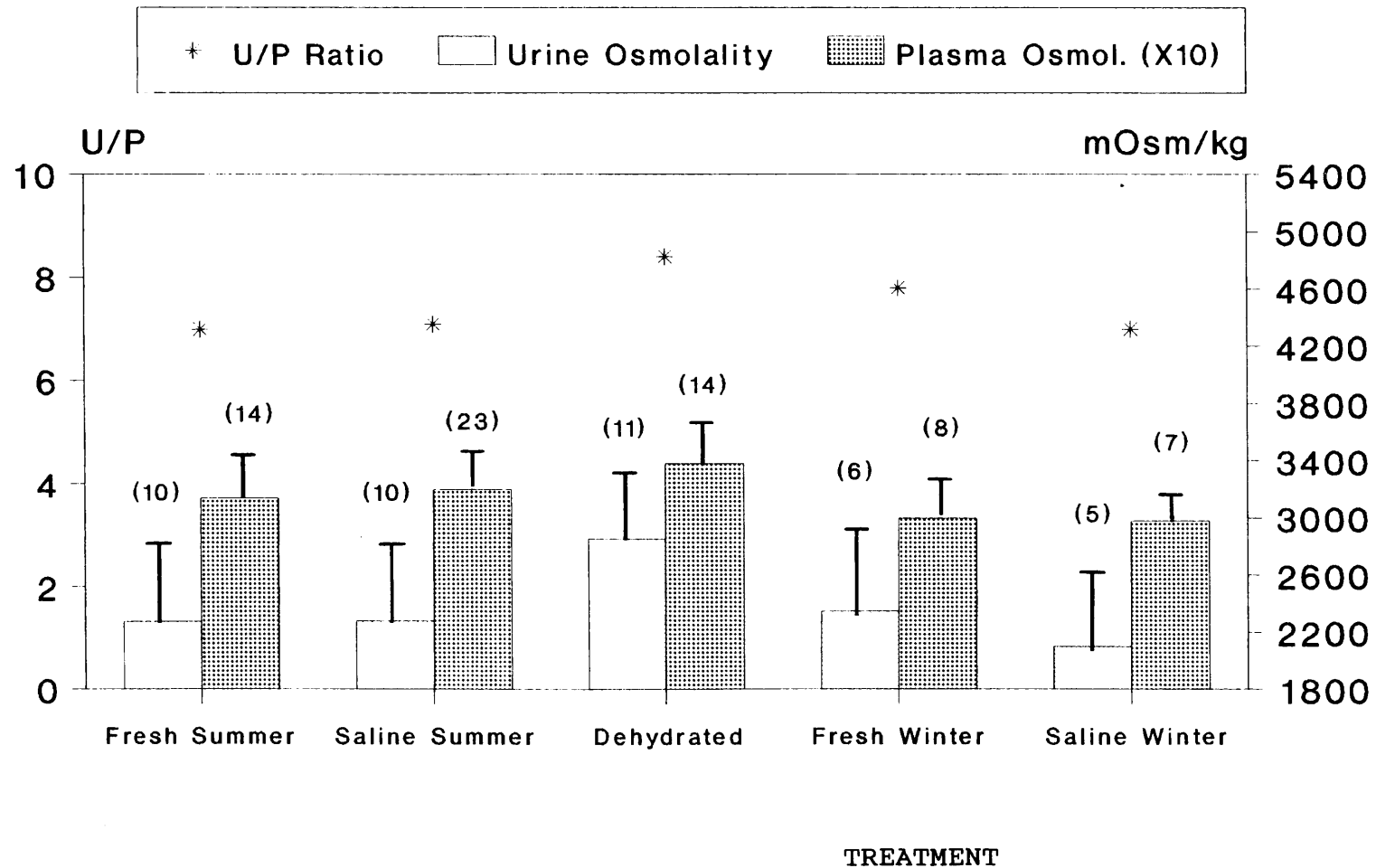
Fig. 7. Urine and plasma osmolality and urine : plasma

ratio (U/P) in gemsbok exposed to different treatments.

Results presented as means ± 1 S.D. Sample size in brackets.

dehydrated animals and those given fresh water in summer.

DISCUSSION AND CONCLUSIONS

Glomerular filtration rate

The lack of significance in differences in GFR between any hydrated treatment suggests that the GFR can be maintained even though differences, such as water quality and temperature, exist in the external environment. This is believed to be a result of intra-renal control or autoregulation (Gottschalk & Lassiter 1974).

Autoregulation may be achieved by varying the constriction of the efferent and afferent arterioles. This ensures that the desired glomerular capillary hydrostatic pressure is maintained, and that the filtration rate remains fairly constant should there be changes in blood pressure (Guyton 1977). Tubuloglomerular feedback is another known autoregulator of GFR. In this case, the macula densa cells of the early distal tubule come into direct contact with the afferent arteriole of the parent glomerulus. It is thought that the concentration of NaCl in the distal fluid is sensed as the fluid passes the macula densa region. An increase in the concentration of NaCl in the distal tubule, leads to a decreased GFR, hence returning the concentration of NaCl in the distal fluid to previous levels (Dantzler 1989).

The extent of changes in GFR and excretion are influenced by many

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factors. Guyton (1977) lists some of the most important as being -
i) the plasma colloid osmotic pressure - an increase would decrease
GFR while increasing tubular reabsorption of water; ii) the
sympathetic input to the kidney - control in this case is usually
by way of the afferent arteriole and constriction of this arteriole
results in reduced glomerular pressure and hence filtration rate;
iii) the arterial pressure - an increase in mean arterial pressure
increases GFR by increasing the glomerular pressure. It also
increases the peritubular capillary pressure, which results in
decreased water absorption and increased urine volume. The net
effect of an increased GFR therefore, is less tubular reabsorption
and hence increased excretion of water and Na. Wesson, Anslow,
Raisz, Bolomey & Ladd (1950) and Smith (1951) believed that changes
in the above variables occur in response to the decline in body
fluids. This appears to be borne out in the present study. In
summer, ECFV was lower in saline water acclimated animals than
fresh water acclimated animals, and lower still in dehydrated
animals. At the same time, plasma Na concentration and plasma
osmolality were higher in saline water acclimated animals and
reached maximums in dehydrated animals. Plasma protein although
similar for fresh and saline acclimated animals in summer, was
higher in dehydrated animals.

The relative rise in the plasma protein concentration due to a
reduced ECFV would result in an increased colloid osmotic pressure
in the blood (Guyton 1977). GFR is proportional to the permeability

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of the filtration membrane and the net filtration pressure, the latter being dependent on the hydrostatic pressure in the glomerulus, the back pressure of the filtrate and the oncotic pressure of the plasma (Gottschalk & Lassiter 1974). The higher plasma protein concentration found in dehydrated animals in summer, would therefore have the effect of increasing glomerular capillary oncotic pressure which in turn would decrease GFR (Vander 1975). GFR was indeed lower in dehydrated animals than in animals acclimated to fresh water in summer. However, Gottschalk & Lassiter (1974) stated that change in the colloid osmotic pressure is not the major determinant of changes in GFR, since such changes could be compensated for by changes in the resistance of efferent and afferent arterioles.

Reduction of GFR during dehydration has been recorded by a number of authors (McCance & Young 1944; Siebert & Macfarlane 1971; Maloiy 1972; Yagil & Berlyne 1976; Yagil & Berlyne 1977; Hewitt, Wheldrake & Baudinette 1981; Etzion & Yagil 1986). Macfarlane & Siebert (1967) found that in dehydrated camels, GFR declined to one-quarter that obtained when hydrated. In rats, Rattus spp., deprived of water, GFR declined by 18% after three hours (Edwards 1982).

There are two advantages of decreasing GFR in summer saline acclimated and dehydrated gemsbok. First, there is a reduction in the amount of fluid presented to the tubules, which would have the effect of reducing the amount of water that could be excreted

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(Maloiy 1972; Gellai, Edwards & Valtin 1979). Secondly, with less fluid in the tubules, tubular reabsorption may be enhanced. Gellai et al. (1979) proposed, and Bakker & Bradshaw (1983) suspected, that a decrease in GFR leads to a reduced flow of fluid in the distal segment which in turn enhances the passive reabsorption of urea into the inner medulla. This would increase the ability of the kidney to absorb water (Rugungazi & Maloiy 1988). In camels, a reduced filtration rate rather than an increased urine concentration has been found during dehydration (Maloiy, Macfarlane & Shkolnik 1979).

Rugungazi & Maloiy (1988) also noted that the ability of the dik-dik to inhabit hot arid lands resulted from a decreased GFR and increased tubular urea reabsorption.

Another result of the reduced GFR would be to decrease the amount of Na filtered, which could lead to a decrease in Na excretion (relative to normal values). Since water largely follows Na, less water would also be excreted. If the ECFV is decreasing, and plasma Na concentration and osmolality are increasing, it is likely that dehydration is occurring. An increased plasma Na concentration and plasma osmolality would tend to draw water into the ECFV from the intracellular compartment, in an attempt to restore this volume loss. Evidence for the loss of intracellular fluid is presented in Chapters 2 and 4.

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The increase in the GFR of saline acclimated animals in winter compared to those acclimated to fresh water in winter, is attributed to a slightly increased ECFV, with a slightly decreased plasma osmolality and plasma protein content.

An increase in GFR in animals exposed to saline water was also reported by Ladd & Raisz (1949), Wiggins, Manry, Lyons & Pitts (1951), Wesson (1957), Earley & Friedler (1965) and Vander (1975). Maloiy (1972) found that when camels were saline loaded (2,75%), GFR increased by 57%. The advantage of an increased GFR for saline acclimated gemsbok in winter, would be to make more electrolytes available in the filtrate for excretion. Hence excretion is enhanced (Wiggins et al. 1951). Secretion of K and H^+ may also be enhanced since the greater the flow rate in the lumen the larger will be the secretion of a substance (Young 1985). Increased flow rate in the tubules may lead to increased water losses since proximal water absorption is passive. However solute free water reabsorption is known to occur in the collecting ducts due to ADH (Gordon 1977).

To conclude, it is apparent that excretion is not only dependent on GFR. This is also recognized by Wiggins et al. (1951), who noted that changes in Na excretion cannot be related solely to changes in the GFR or ERPF. Other mechanisms, some of which are discussed below, are definitely involved.

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Tubular reabsorption

A major part of the control of excretion is in fact carried out in the tubule. In mammals, absorption of a large part of the Na and Cl filtered load occurs in the tubules (Dantzler 1976, in Dantzler 1989), and more than 99% of the filtered Na may be absorbed in the tubules (Dantzler 1987, in Dantzler 1989). Of this, between 60 - 80% is absorbed in the proximal tubule (Giebisch & Windhager 1964). Knowledge of the pathways of Na reabsorption in the distal and collecting tubules is incomplete (Vander 1975).

Tubular reabsorption is affected by factors such as surface area of the lumen, tubular concentration of electrolytes and the amount of poorly dissolved solids present. In addition, the permeability of the luminal border of the cell may be altered by a number of factors including the presence of hormones (De Wardener 1973).

It would appear from Table 4, that the filtered loads of Na and Cl in dehydrated animals are considerably different from those of animals acclimated to fresh and saline water in summer. The advantage in this is that a reduction in filtered load decreases the total Na in the filtrate, and with less Na in the distal tubule there may be an effect on the macula densa transport of Na and hence on renin release (Guyton 1977). This in turn would result in increased absorption from the tubules (Guyton 1977), which would benefit the dehydrated animal.

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Effective renal plasma flow and effective renal blood flow

Changes in the amount of blood and plasma flowing through the kidneys are brought about by many factors, including changes in blood pressure. Much of the stabilization of these changes is undertaken in the kidney itself, and is controlled by sympathetic innervation and humoral factors like angiotensin, adrenal steroids, vasopressin, and prostaglandins (Koushanpour 1976). This control, as for GFR, may have resulted in the constancy of ERBF seen in hydrated animals in the present study.

Changes in GFR were accompanied by changes in the ERPF, a finding also reported by Macfarlane & Siebert (1967, in Maloiy 1972). The reason being that hydration state affects ERPF, via its effects on ECFV, which affects glomerular pressure and therefore GFR.

Yagil & Berlyne (1978) found that ERPF declined in early and mid-summer when camels were dehydrated. In the present study ERPF declined during dehydration by almost 50% compared to that found in fresh water acclimated animals. Dehydrated animals are also reported to have reduced ERPF's or RBF's by Maloiy (1972), Hewitt et al. (1981) and Etzion & Yagil (1986). Macfarlane et al. (1961) found that following three days of dehydration in Merino sheep, RPF had fallen from 460 - 786 to 420 - 600 ml/min. After five days it had fallen to 240 - 336 ml/min.

In the present study ERPF decreased in saline acclimated animals in

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both winter and summer. Wiggins et al. (1951) found an increase in RPF when humans were saline loaded although not significantly so. Potter (1963) found no change in the RPF of sheep given 1,3% saline.

Sullivan (1974) reported that an increased RBF would increase the loss of water from the system. This being due to the decreased peritubular capillary colloid osmotic pressure that results from an increased ERPF (Vander 1975). The decrease in ERPF values in animals acclimated to saline water, compared with those acclimated to fresh water, would tend to reduce the loss of water from the system. This is more pronounced in dehydrated animals. However, since change in peritubular colloid osmotic pressure is a function of the FF (Sullivan 1974), the FF should be considered.

Filtration fraction

Filtration fraction in humans is usually around 16 - 20% (Koushanpour 1976). Hewitt et al. (1981) found the FF increased in short and long term dehydration. This was also found in the present study, where a maximum value of 21% was recorded in dehydrated gemsbok. The reason for this is that GFR varies less than ERPF, and when there is a decrease in the systemic blood pressure (as would probably occur when ECFV decreases in dehydration) GFR falls less than the ERPF as a result of efferent arteriolar constriction (Ganong 1977).

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Changes in the FF are important since changes in peritubular hydrostatic pressures and oncotic pressures result from such changes (De Wardener 1973; Yagil & Berlyne 1976). The reason for this is that protein is not filtered at the glomerulus. As the ratio between blood arriving at the glomerulus and that filtered by it changes, a variation in the relative amount of protein delivered to the peritubular capillaries results. This, for example, may increase the proximal tubular reabsorption of Na and water (Giebisch 1979).

The values for FF found in the present study in winter are lower than those recorded in summer, and may be indicative of the lack of water or heat stress in winter. The high value obtained for animals acclimated to fresh water in summer could conceivably be an artifact of the large variation found in ERPF values on this treatment.

Urine osmolality

Urine osmolality changes are well recorded in the literature when animals have been exposed to dehydration and saline loading.

Hewitt et al. (1981) found that urine osmolalities were always higher in dehydrated than hydrated animals. Dehydrated camels had osmolalities of 3000 mOsm/kg H_2O (Maloiy 1972) and 3100 mOsm/kg H_2O (Macfarlane & Siebert 1967), and Etzion & Yagil (1986) found the urine osmolality increased in the camel from 620 mOsm/kg H_2O when hydrated to 2100 mOsm/kg H_2O when dehydrated. Dehydrated sheep and

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cattle <u>Bos</u> <u>spp.</u>, had osmolalities of 3800 mOsm/kg H_2O and 2600 mOsm/kg H_2O, respectively (Macfarlane & Howard 1972). In the present study, dehydrated gemsbok had a mean osmolality of 2849 mOsm/kg H_2O, which was significantly different to that found in fresh water acclimated animals in summer.

Maloiy (1970) noted an increase in urine volume from around 0,7 - 1 l/day to 3 - 5 l/day in the donkey given 0,25 - 3% NaCl solutions. In humans, administration of 500 ml of 3,6% NaCl or 1,2% KCl resulted in an increase in urine flow rates, even when dehydrated (McCance & Young 1944).

The ability of the camel to survive when ingesting large quantities of electrolytes has been attributed to its ability to excrete concentrated urine. Chloride is excreted in concentrations up to 1000 mEq/l (Maloiy 1972). By comparison the donkey could only maintain weight on solutions below 1% NaCl and it could only produce a maximum osmolality of 1500 - 1600 mOsm/kg H_2O, although plasma Cl concentration remained constant and urine Cl concentration reached 680 mEq/l (Maloiy 1972). Sheep also increased the excretion of Na and Cl on saline water (Potter 1963).

Donaldson & Edwards (1981) found that after five days of saline water treatment (2% NaCl) in gerbils, <u>Meriones</u> <u>unguiculatus</u>, the urine osmolality was not significantly different from controls, even though there were large increases in Na and Cl. This is

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similar to the present study, and may indicate that there were changes in the composition of the electrolytes making up the urine osmolality. Indeed, Macfarlane, Morris & Howard (1956) found that less than half the urine osmolality could be accounted for by K and Na. The other substances making up the osmolality were not mentioned. Maloiy (1973a) also found that the total sum of urinary cations and anions did not balance with total osmolality. It is not known what substances, other than those mentioned, were responsible for the urine osmolality in the present study.

U/P ratio

Urine osmolality to plasma osmolality ratios are an indication of the concentrating ability of the kidney. Camels, eland (<u>Taurotragus oryx</u>), sheep, goats and other antelope excrete highly concentrated urine with a U/P ratio of 6 - 8 when dehydrated (Maloiy 1973b). In the donkey a value of only 4,5 was recorded (Maloiy 1970).

Maloiy (1972) found that the U/P ratio in camels subjected to either dehydration or saline loading increased from 5 to 8. McCance & Young (1944) suggested that in humans, the decline in U/P ratio from 3,1 to 2,1 after saline treatment, indicated that changes in the regulating mechanisms had occurred.

The U/P ratios in gemsbok were comparable to other arid adapted species. The increase in the U/P ratio of dehydrated animals is believed to be caused primarily by an increase in urine osmolality

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relative to plasma osmolality (Fig. 7.). There is no reasonable explanation for the high value of the U/P ratio in animals acclimated to fresh water in winter. It appears that plasma osmolality is reduced while urine osmolality is similar to that found for animals in summer. The reason for this is unknown.

In conclusion, the effects of saline water on GFR, ERPF and FF were not as marked as those of dehydration. However this study indicates that 1,8% saline drinking water approaches the upper limit that gemsbok can tolerate. In dehydration, renal function changed dramatically in the direction of increased water conservation. Since it is conceivable that a reduction in GFR is an adaptation to meet the threat of body desiccation (Khan, Sasidharan & Ghosh 1979), then the renal response to dehydration may be considered as an important part of the gemsbok's adaptation to life in arid regions.

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CHAPTER 4

BODY FLUID COMPARTMENTS

INTRODUCTION

Body water comprises approximately 73% of the lean body mass in man and most other species, yet it is not distributed uniformly throughout the body. It is, in fact, compartmentalized. The two main body fluid compartments are the intracellular fluid, and the extracellular fluid. The extracellular fluid is composed of the vascular and interstitial fluids.

The intracellular fluid is defined as that fluid present within the cells, and, in humans and some other species studied, the intracellular fluid comprises approximately 35% of body mass. The vascular fluid is found only within the blood circulatory system, and accounts for approximately 7% of body mass, while the interstitial fluid (approximately 19,5% of body mass) comprises the lymph, the fluids in dense connective tissues, cartilage and bone and the cavity fluids. The latter are also known as transcellular fluids and comprise the digestive, cerebrospinal, pleural, intraocular, peritoneal and synovial fluids (Koushanpour 1976).

Distribution of water within these compartments is ultimately

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dependent on osmolality (Spurlock, Landry, Sams, McGuirk & Muir 1985). Since total body osmolality is proportional to the total body Na plus total body K, divided by total body water (TBW), it follows that osmolality is affected either by changes in the electrolytic composition or fluid volume (Ganong 1977). It is known that the movement of cations is restricted to some extent by the cell membrane, and therefore osmotic regulation is probably brought about by the movement of water (Darrow & Yannet 1935).

In animals subjected to some abnormal physiological condition, such as disease, overhydration or dehydration, changes in both fluid volumes and compositions are likely to occur. If animals are thermally dehydrated, loss of water from the fluid compartments is usually accompanied by an increase in the osmolality of body fluids (McKinley et al. 1983). It is generally believed that the water source available to animals that lose body fluids due to thermal influence is the blood fluid (Horowitz & Borut 1970). Yet in many cases the total loss of fluid exceeds that of the blood plasma, indicating loss from other compartments. Various studies have shown that the water compartments contribute differentially to the fluid loss, and this differs between species (Horowitz & Borut 1970). In dogs (Elkington & Taffell 1942, in Macfarlane et al. 1961; Thrasher et al. 1984), there is equal withdrawal of fluid from the intra- and extracellular fluids. In ruminants it is not clear whether there is equal withdrawal of cellular and gut water during dehydration, although in the camel, much use of the gut water is

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made (Macfarlane <u>et</u> <u>al.</u> 1956). Since death may be associated with loss of circulatory fluid, the conservation of circulatory fluid and the reliance on other compartments during water stress is seen as an important adaptation in the survival of arid dwelling species.

Therefore, if an assessment of the ability of the animal to cope with water stress is to be made, it is necessary to determine the extent and nature of the changes in the body fluids.

MATERIALS AND METHODS

Introduction

The determination of a body fluid compartment may be achieved using the dilution principle. A known quantity of a specific tracer substance is injected into the system and its resulting concentration is an indication of the size of the compartment measured. The tracer substance used to determine specific body water compartment volumes must satisfy the following conditions - i) it must distribute only within the compartment of interest; ii) it must not be metabolized nor eliminated from the body water; and iii) it must be non-toxic and the determination of its concentration must be accurate (Holleman, White & Luick 1982).

Data analysis involves the resolution of a semi-logarithmic plot of plasma tracer concentration with respect to time. For this to be

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accurate, time dependent processes such as mixing in the blood and then distribution of the substance to the compartment of interest, must be achieved. This is represented graphically by an initial non-linear section which suggests the mixing phase and is followed by a more linear section as the tracer substance is distributed in the compartment being tested. Extrapolation of the linear component through the y-ordinate provides the theoretical concentration at t = 0, assuming instantaneous and complete mixing. This is simplistically represented by the following equation -

volume of body fluid =   quantity of tracer injected
      compartment   concentration of tracer in blood at t=0

(Equation 1)

The choice of tracer substance and the method employed is obviously critical to the accuracy of the determination, and a brief discussion follows.

Total body water

The most obvious method of determining the body water content of an animal would be to desiccate it so that the change in weight would represent the water content. This can rarely be justified, and is often not practical. The use of a tracer substance that can accurately determine this fluid space is indicated.

Tritium, an isotope of hydrogen, appears suitable when bonded to a

stable hydrogen and oxygen atom to form tritiated water (HTO). Although radioactive, it is thought to be safe in tracer concentrations since it emits low energy $\underline{b}$ particles.

In determining TBW, various assumptions are made. First, that the subject is in a steady state of composition i.e. TBW, water inflow and water outflow are stable. Second, that the TBW of an animal is described by a single compartment through which the tracer distributes rapidly and uniformly. Third, that the substance used is not incorporated into other body constituents, and that it is lost from the body only as that substance. Fourth, that the concentration of substance lost from the body is the same concentration as that within the body. Lastly, that the substance does not enter the body through some other means such as the respiratory surfaces or the skin.

If all the assumptions are met then : water outflow = water inflow + metabolic water production (Holleman $\underline{et}$ $\underline{al.}$ 1982). Whether all these assumptions are met when using HTO is not certain. That inaccuracies do occur with HTO is evident. Many authors have reported error in the use of HTO. Haines, Macfarlane, Setchell & Howard (1974) and Hewitt $\underline{et}$ $\underline{al.}$ (1981) found that HTO underestimated TBW. However Tisavipat, Vibulsreth, Sheng & Huggins (1974) reported an overestimate of 8% in the rat. Holleman & Dieterich (1975) showed that the 95% confidence interval for an individual estimate of TBW using HTO was $\pm$ 9,8% of the value

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determined by direct analysis. Their study showed a 10%
overestimation of true volume for TBW using HTO.

The discrepancy between the use of an indirect and direct method is
thought to be caused by a number of factors. First, Holleman et al.
(1982) point out that TBW overestimation when using HTO, is due to
isotopic exchange of hydrogen with labile hydrogen atoms in the
non-water constituent of the body. This may amount to a 5%
overestimate of TBW, although Sheng & Huggins (1971) found this to
be only 0,5 - 2% of the overestimation.

Second, slow exchange of HTO also occurs with the organic
components of the body such as bone and teeth, and introduces some
error in TBW determinations. Furthermore, it is not certain whether
HTO exchanges with skeletal muscle water, 5% of which may be bound
(Lewis & Phillips 1972).

Other errors are introduced by the isotopic fractionation of water.
This may occur in evaporation, since HTO is slightly heavier than
water and remains behind during evaporation. Holleman et al. (1982)
also pointed out that HTO leaves the body water pool more slowly
than water. Similarly, error is caused by exchange of water at the
skin and lung surface.

It is apparent that there may be an inherent error in this
technique. However, since total body desiccation was undesirable,

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HTO was chosen to measure TBW. It was assumed that HTO space represented TBW space.

Extracellular fluid volume

A number of different substances have been used in determining ECFV, but all have drawbacks. Sodium thiocyanate (NaSCN) is widely used as a non-radioactive tracer to determine the ECFV.

NaSCN tends to correlate well with the Bromine-82 space which is considered a good measure of ECFV (Kohn, Muir & Sams 1978). Huang & Bondurant (1956) reported that it gave the same results as sulphur-35 and chloride, both good indicators of ECFV (Lavietes, Bourdillon & Klinghoffer 1936). Furthermore, these authors found that NaSCN correlated well with known changes in ECFV, indicating that NaSCN is, in all likelihood, extracellular. In addition, the distribution of NaSCN is similar to sucrose and inorganic sulphate – and these do not enter cells. The advantage of NaSCN over sucrose and sulphate, is that it is not excreted as rapidly as sucrose or sulphate (Lavietes et al. 1936). This allows for a more complete distribution throughout the body. Sucrose is excreted so rapidly that error can be caused if it is injected in concentrated solutions, while sulphate distribution is uncertain between serum and transudates (Lavietes et al. 1936).

Kohn et al. (1978) did however find that NaSCN penetrated cells when body temperature increased, giving rise to concern over what

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fluid volume NaSCN measures. Denny & Dawson (1975) stated that NaSCN was not found in the gastro-intestinal tract, and they suggested this space must be included in the intracellular space. Conversely, Crandell & Anderson (1934, in Lavietes et al. 1936) found that NaSCN entered all gastric spaces at the same concentration as in the serum. They found that sucrose did not.

Inulin, another tracer used in ECFV determinations, has an advantage over NaSCN as a tracer substance in that it is an elongated molecule which may be hindered from entering cells. In the case of NaSCN, the semi-permeable cell membrane may not be effective in preventing entry. Hix, Underburg & Hughes (1959) found the NaSCN space to be larger than the inulin space in the domestic dog and man. This they attributed to the fact that NaSCN measures a more dynamically functional space, the physiological volume, as opposed to inulin which measures the anatomical volume. As such, NaSCN is a more realistic measure of complete ECFV.

It is generally believed that NaSCN is a good but not excellent measure of ECFV (Kohn et al. 1978; Carlson, Harrold & Rumbough 1979 in Kami, Merritt & Duelly 1984). However Darrow & Hellerstein (1958) noted that it was unlikely that any substance would reliably measure ECFV. NaSCN was used in this study primarily because it had the advantage of being safe; could be injected in small concentrated aliquots; and it equilibrated rapidly hence minimizing errors. The fluid compartment measured by NaSCN was assumed to be

equivalent to the ECFV.

Plasma volume

Until recently, Evans Blue was used extensively as a tracer to measure plasma volume. It has now been superseded by the radio-immuno serum albumin (RISA) method.

Huang & Bondurant (1956) found no difference between Evans Blue, or T-1824 as it is also known, and RISA in the measurement of plasma volume. However Evans Blue has been criticized by some authors as being inaccurate. Zizza & Reeve (1958) found that Evans Blue overestimated plasma volume by 9% in laboratory rabbits, Oryctolagus cuniculus, while an overestimate of 6% was reported by Reese & Haines (1978). Furthermore, Zizza & Reeve (1958) reported that Evans Blue was distributed in a larger volume than Iodine-131 albumin in rats. They suspected that Evans Blue had binding sites outside of the vascular space. Using the Evans Blue / haematocrit method, Gregerson (1944) found the blood volume to be 103% of that found using the RISA method. The differences are thought to be due to the fact that there is a varied ratio between the overall cell percentages in the circulatory system; venous blood haematocrit being slightly higher than whole body haematocrit (Gregerson 1944).

A possible error of the size presented above, was considered to be small, and acceptable for the present study since the need for a non-radioactive substance that could be readily assayed, was

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fulfilled by Evans Blue. Evans Blue space was assumed to equal plasma volume.

Experimental application

Total body water

Following the collection of a blood sample. for background radioactivity determination, approximately 0,7 mCi/kg body weight HTO (Amersham International, Amersham, U.K.) was injected into the jugular vein, with the syringes being weighed before and after injection. Dehydrated animals were included in the TBW determinations. Samples of the injected solution were retained for determination of radioactivity. Blood samples were collected as described in Chapter 1, and following centrifugation, plasma was removed and kept frozen at -10°C until analysed for radioactivity.

Samples, including the background samples, were analysed for radioactive decay in a Packard 1500 Tri-carb Liquid Scintillation Counter (Packard Instrument Co., Illinois, U.S.A.) after adding 4 ml Hionic Fluor liquid scintillation cocktail (Packard Instrument Co., Illinois, U.S.A.) to 0,5 ml of plasma. This cocktail was recommended for the analysis of highly concentrated salt or alkaline samples, and was well suited to the plasma samples. Results were presented as disintegrations per minute (dpm) and quenching was accounted for by internal standards. Standards were diluted 1:10000 and 0,5 ml were added to 4 ml scintillation cocktail and the sample counted.

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Data were analysed by plotting dpm semi-logarithmically against time which generated a plasma HTO disappearance curve (background radiation was subtracted from sample counts). The linear portion was identified and a least squares linear regression line fitted to these points. This line was extrapolated to cut the y-ordinate and provided a value for the concentration of the tracer at time zero. Calculation of the TBW was based on the mathematics of the dilution principle.

Extracellular fluid volume

One hundred millilitres of 0,9% sterile saline was heated to near boiling point and 10 g NaSCN slowly added. The solution was vigorously shaken for 10 min and cooled. It was then passed through a 0,25 μm bacterial filter (Millipore Corp., Bedford, Ma., U.S.A.) and stored in sterile containers.

Approximate dosage was 15 mg/kg body weight. Syringes were weighed before and after injection of the tracer. Prior to injection of the tracer via an indwelling jugular catheter, a blood sample (blank) was drawn. Blood sampling was carried out at specified intervals (see Chapter 1).

Each blood sample was centrifuged, the plasma collected, and 1 ml from each sample was placed in a test tube. A 20% trichloroacetic acid solution was prepared and 6,5 ml of this was added to each tube. Following mild shaking, each tube was allowed to stand for 10

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min before being centrifuged at 2000 rpm for 10 min at room temperature. A ferric nitrate/nitric acid solution was made by dissolving 80 g ferric nitrate in 250 ml 2N nitric acid, which was then made up to 500 ml with distilled water. Five millilitres of the supernatant and 5 ml of the ferric nitrate/nitric acid solution were mixed in complete darkness, and the resulting solution was assayed within 10 min. One millilitre of the blank and 1 ml of standard (diluted 1:10000) were also processed as described.

Determination of the concentration of NaSCN in plasma and an aliquot of the injected dose, was performed using a spectrophotometer set at wavelength 460 nm. The sample blank was used to set zero absorbance.

A calibration curve was constructed using known concentrations of NaSCN dissolved in water and analysed in the same manner as that of the samples. The Beer-Lambert Law was obeyed in the range of concentrations used in this study, and a least squares linear regression analysis of concentration versus absorbance was performed ($r^2 = 0,99$).

Sample absorbance values were then read against the calibration curve and the concentration of NaSCN determined. A graph of concentration versus time was plotted and the linear section of the graph was identified. These points were subjected to least squares linear regression analysis and the line extrapolated back through

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the y-ordinate to provide the theoretical concentration of NaSCN in the fluid space at time zero.

Equation 1 was used to calculate ECFV.

Plasma volume

Evans Blue solution was made by adding 400 mg Evans Blue to 100 ml 0,9% sterile saline. The mixture was gently heated to 30°C and stirred for 5 min. It was then filtered through a sterile 0,25 µm bacterial filter (Millipore Corp., Bedford, Ma., U.S.A.) into a sterile container. An approximate dosage rate of 0,4 mg/kg body weight was used, and syringes were weighed before and after injection to ascertain precise quantities of injected substance.

A pre-injection blood sample (blank) was drawn before injection of the Evans Blue solution into the jugular catheter. Blood sampling is described in Chapter 1.

Following centrifugation at 3000 rpm for 20 min at room temperature, plasma was removed from each sample. The determination of Evans Blue in plasma was based on the spectrophotomic determination of its absorbance at 620 nm. The sample blank was used to set zero absorbance.

A calibration curve was constructed using five known concentrations of Evans Blue solutions. The absorbance at each concentration was

plotted and a least squares linear regression analysis of concentration versus absorbance was performed ($r^2=0,99$). Evans Blue obeyed the Beer-Lambert Law in the range of concentrations used in this study. Sample values were read against this curve and the concentration of Evans Blue determined.

A graphical plot of concentration versus time was then constructed, and the linear component of the, plasma disappearance curve was obtained. These concentration values were regressed (least squares linear regression) against time and a straight line was generated and extrapolated to cut the y-ordinate. Theoretically this point represents the concentration of Evans Blue in the plasma at time zero.

The concentration of the injected dose was calculated by diluting it 1000 times and determining the absorbance value. This was converted to a concentration value as described above. Finally the volume into which the Evans Blue was distributed was calculated using Equation 1.

Interstitial fluid volume (ISFV) and Intracellular Fluid Volume (ICFV).

ISFV was calculated by subtracting plasma volume from ECFV and the ICFV was calculated from the subtraction of ECFV from TBW.

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RESULTS

TBW

Equilibration of HTO usually occurred within 110 min of injection in both hydrated and dehydrated animals. The straight line generated from dpm values obtained post-equilibration, yielded lower r^2 values (r^2= 0,41) in dehydrated gemsbok as compared with hydrated animals (r^2= 0,69). This resulted from greater scatter in the plasma HTO decay curve (Fig. 8.).

The TBW of animals in summer was greater than that in winter. In summer, fresh water acclimated animals (n = 13) had a mean TBW content (expressed as percentage of body weight) of 69,4 $\pm$ 9,4%, compared to 66,2 $\pm$ 8,6% in saline acclimated animals (n = 14). This declined to 65,8 $\pm$ 10,2% and 61,0 $\pm$ 11,0% in fresh (n = 6) and saline (n = 4) water acclimated animals, respectively, in winter. When dehydrated (n = 10), a water content of 61,9 $\pm$ 2,7% was recorded.

To account for metabolic size differences TBW was also calculated as body weight^{0,82}, and the results are displayed in Fig. 9. No significant differences were recorded.

ECFV

The results are shown in Fig. 10. and are scaled to body weight^{0,82}. The ECFV of dehydrated animals differs significantly from that of

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(A)

Dpm (×1000)

(B)

Dpm (×1000)
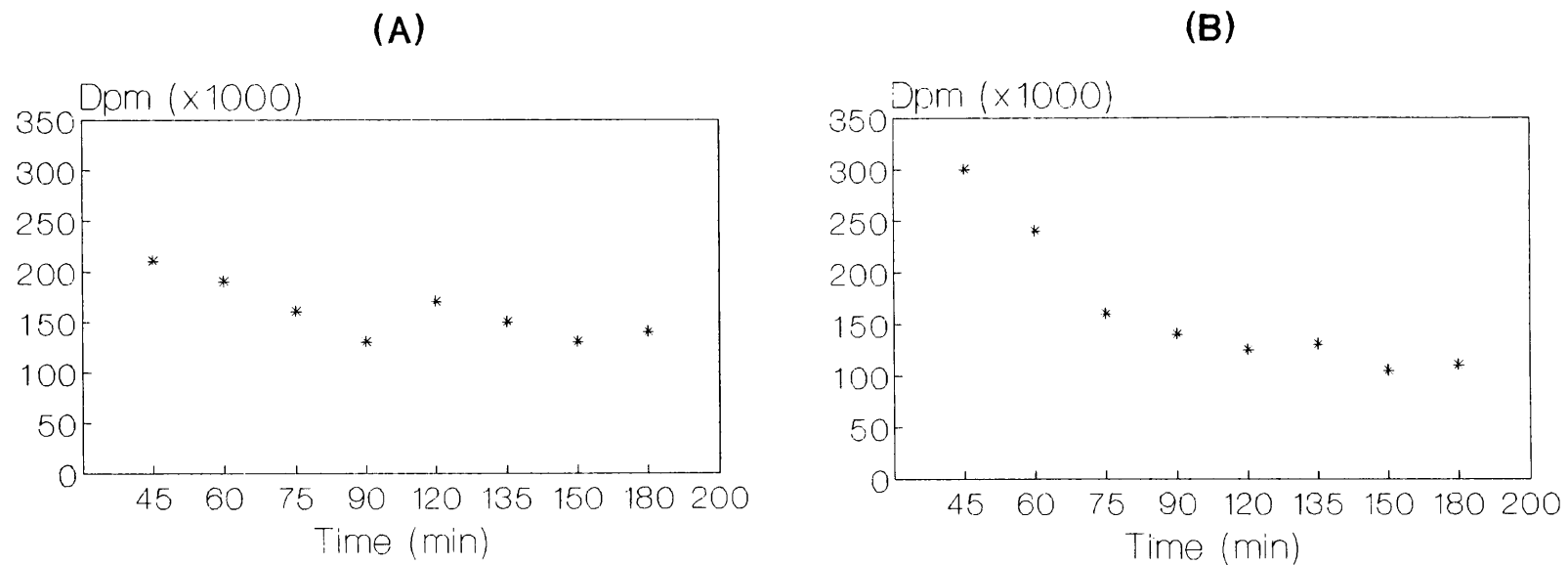
Fig. 8. Representative example of plasma tritium concentration versus time in (A) dehydrated and (B) hydrated gemsbok.
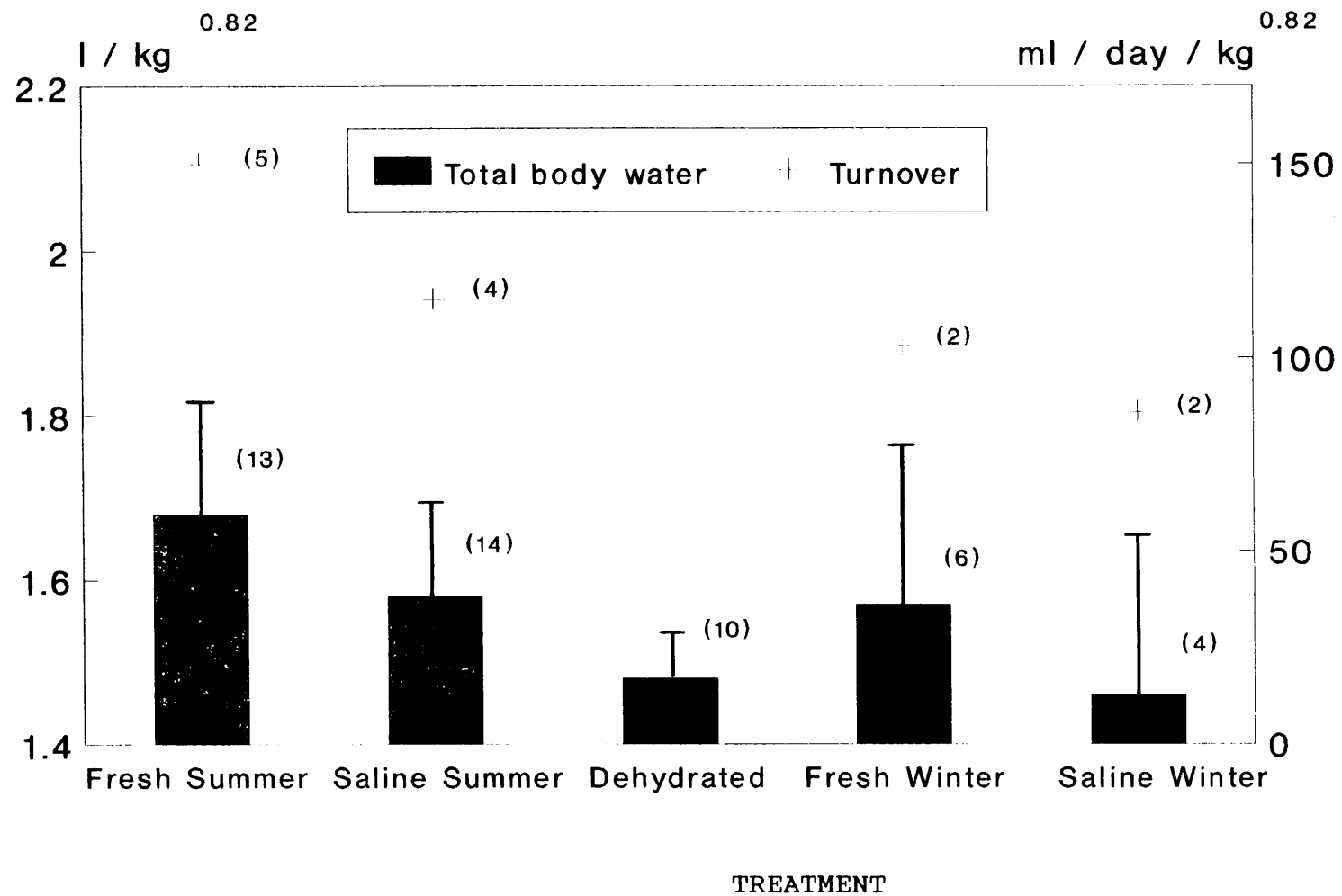
Fig. 9. Total body water versus water turnover rate in gemsbok exposed to different treatments. Results presented as means + 1 S.D. Sample size in brackets.

animals acclimated to fresh water in summer.

ISFV

Total ECFV was divided into its component parts in order to determine changes within compartments. The results were scaled to body weight ^{0,82}, and are presented in Fig. 11. Dehydrated animals had the lowest ISFV found in any treatment, and these were significantly different from animals acclimated to fresh water in summer. No other values differ significantly at this level.

Plasma volume

Plasma volumes scaled to body weight ^{0,82} are shown in Fig. 11. No values differed significantly between any treatments. Plasma volume versus plasma protein concentration is shown in Fig. 12., and will be discussed later in this chapter.

ICFV

ICFVs are presented in Figs. 10 and 11. No significant differences were found between any treatments.

DISCUSSION AND CONCLUSIONS

Total body water

Equilibration of HTO with body fluids is crucial if accurate results are to be obtained. The distribution of an isotope from a

74
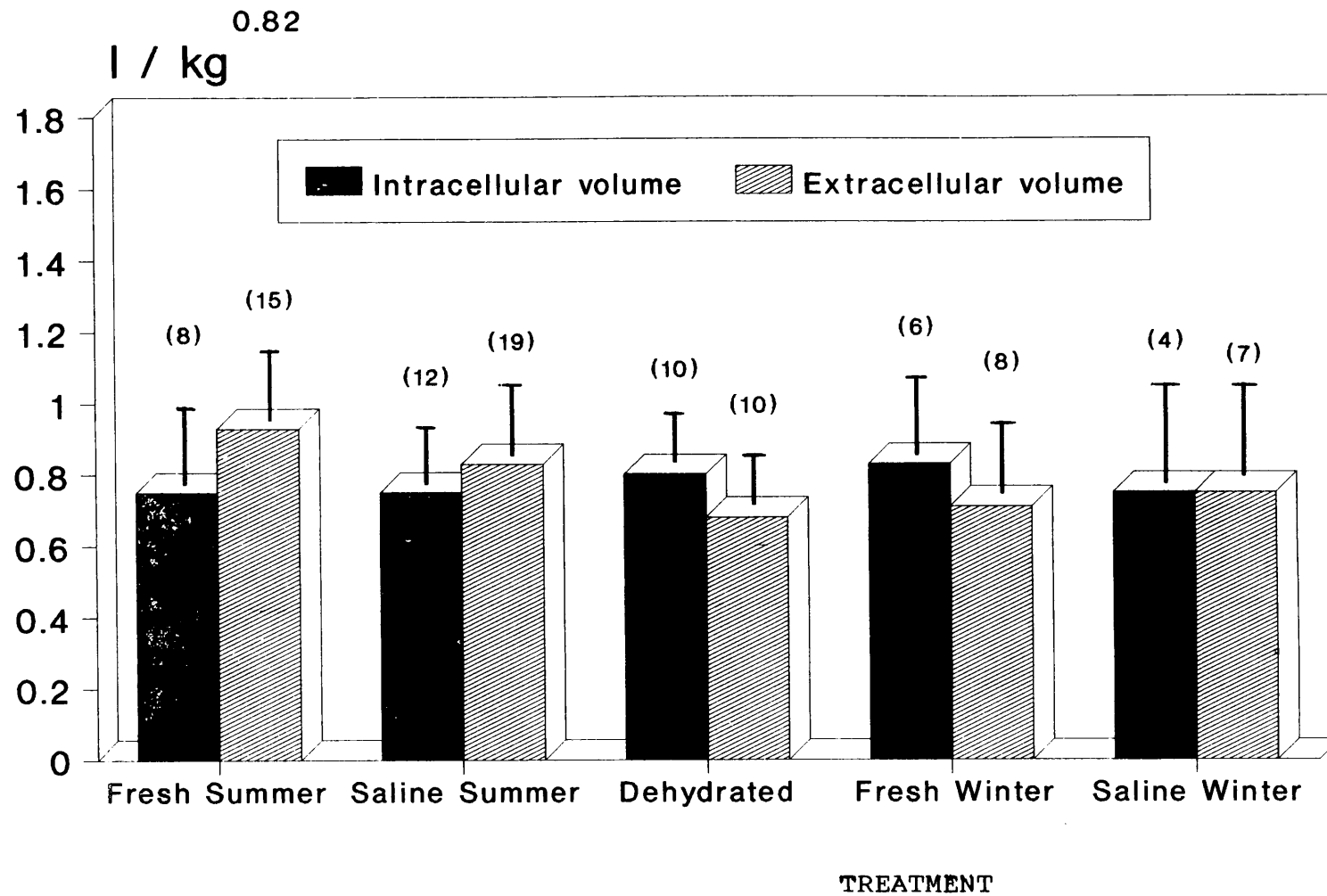
Fig. 10. Intracellular and extracellular fluid volume in gemsbok exposed to different treatments. Results presented as means + 1 S.D. Sample size in brackets.

0.82

l / kg

■ Intracellular volume	▨ Interstitial volume	□ Plasma volume

1.2

(8)
(15)
(12)
(19)
(6)
(10)
(4)
(8)
(7)

1

(19)
(19)
(10)
(8)
(7)

0.8

0.6

0.4

0.2

0

Fresh Summer   Saline Summer   Dehydrated   Fresh Winter   Saline Winter
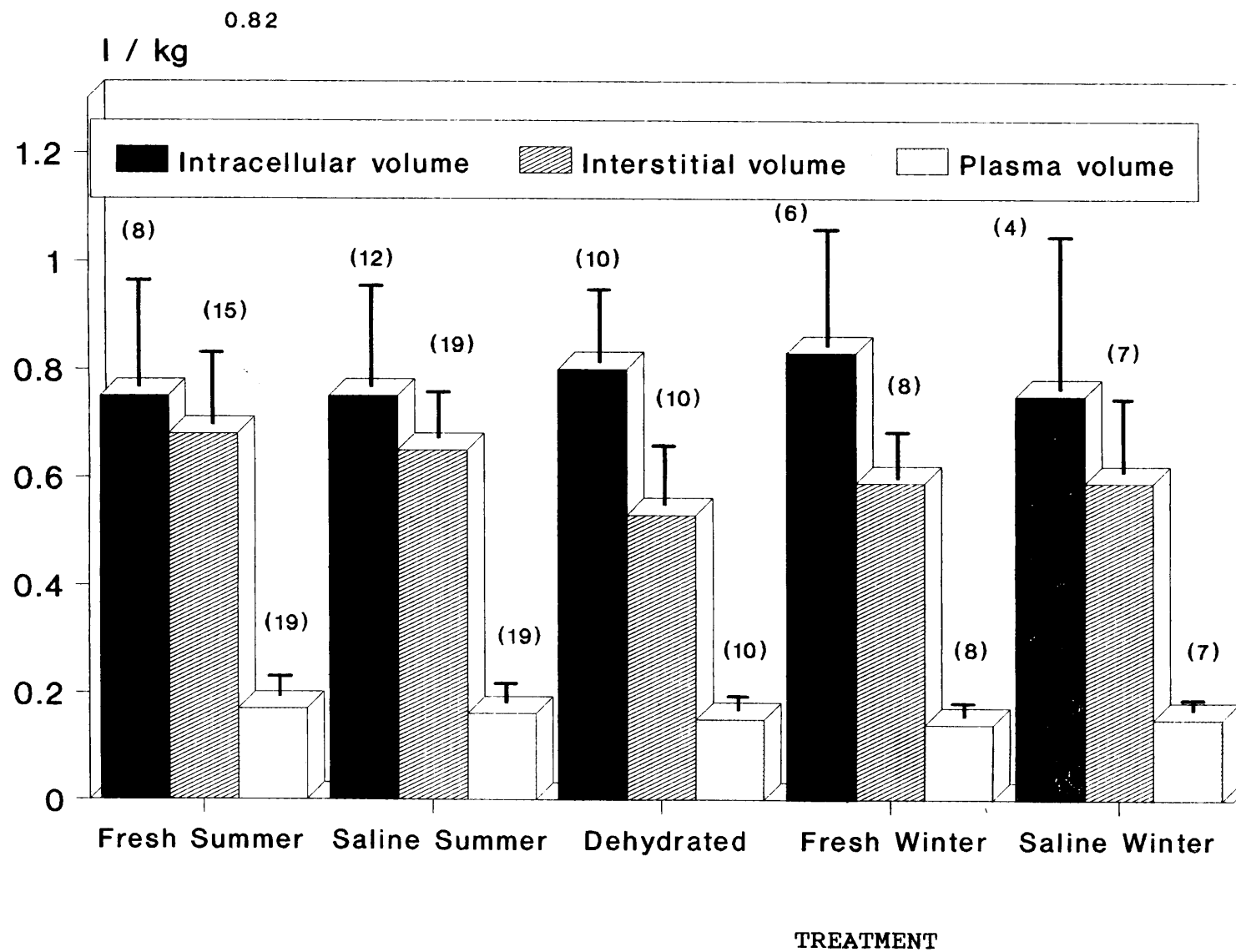
TREATMENT

Fig. 11. Body fluid volume distribution in gemsbok

exposed to different treatments. Results presented as
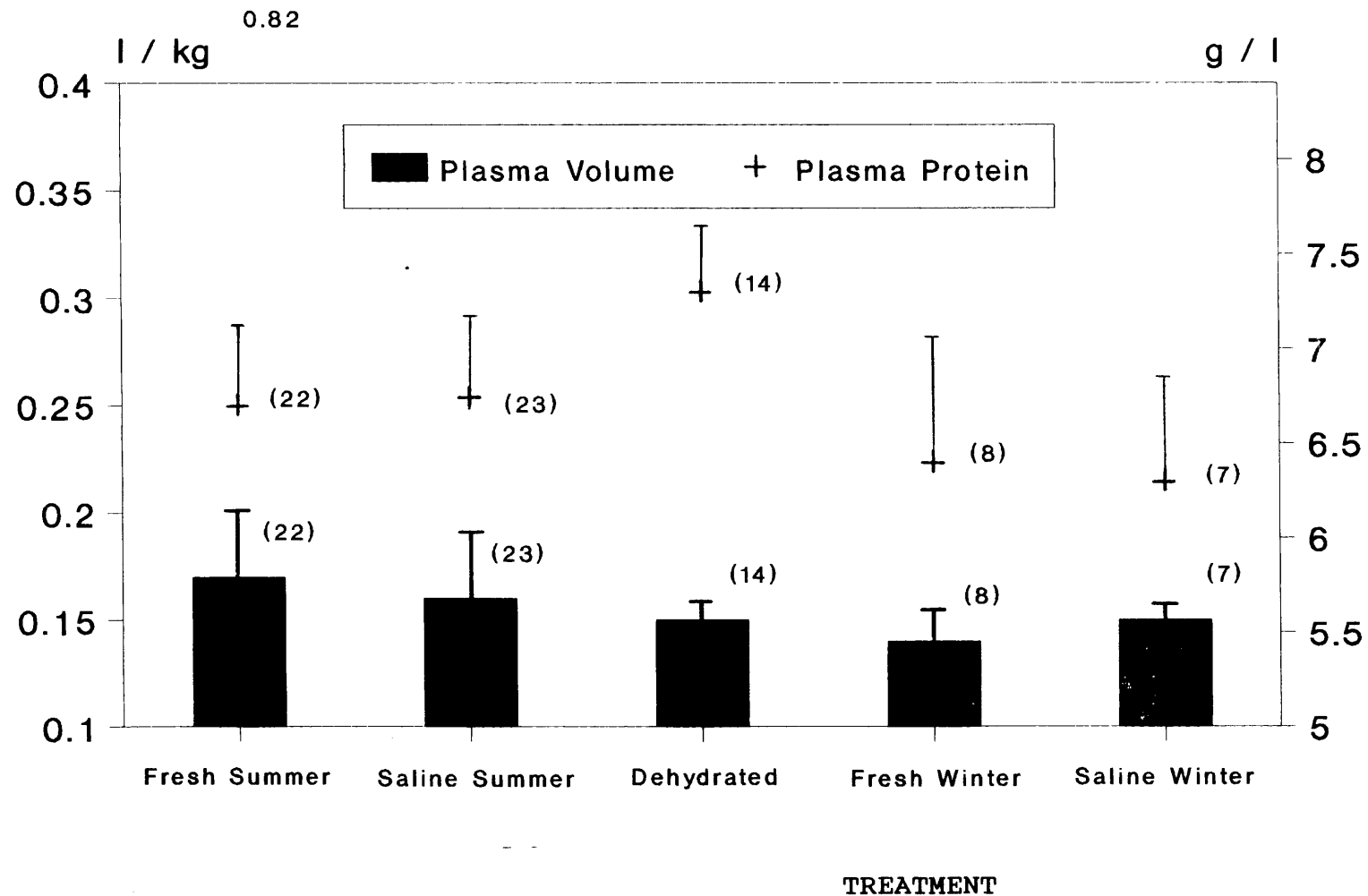
Fig. 12. Plasma volume versus plasma protein concentration in gemsbok exposed to different treatments. Results presented as means + 1 S.D. Sample size in brackets.

venous injection site to body tissues is a function of both transport and diffusion of that isotope from the circulation through the interstitium and into the cells (Coleman, Manning, Norman & Guyton 1972). This may be neither uniform nor rapid, since the peripheral circulation is heterogenous rather than homogenous. Thus, time to equilibration depends on transport of blood to poorly perfused regions (Coleman et al. 1972). The time taken for this to occur is difficult to ascertain as it differs between species and even within individual animals and studies.

Moore, Hartsuck, Zollinger & Johnson (1968, in Lewis & Phillips 1972) found that HTO equilibration may occur within 30 min in man. King & Finch (1982) found that HTO equilibrated with body fluids in Zebu steers, Bos indicus, 1 - 2 h after injection, although it took 2,5 - 3,5 h in calves (Lewis & Phillips 1972).

The rapid equilibration in gemsbok was thought, at first, to be erroneous, implying that incomplete equilibration of HTO with body fluids had occurred. Shumway, Trujillo, Bennett, Mathews & Asplund (1956, in Till & Downes 1962) stated that although HTO equilibrated with body fluids in steers within 110 min, it only equilibrated with rumen fluid 2 - 5 h after injection. Failure to ensure that equilibration has occurred with gut fluids is cited by Smith & Sykes (1974) as one of the main reasons for inaccuracy using HTO. These authors found that 8 h were required for complete equilibration to occur between gut and other body fluids.

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However, if we assume that water in the alimentary tract may account for 30% of body water (Smith & Sykes 1974), and that except for the gut fluids, complete equilibration occurred throughout the body, then the TBW should have been underestimated by up to 30%. In the unlikely event that this was so, then TBW in the gemsbok would account for approximately 90% of body weight. This is somewhat unlikely, and therefore it appears that complete, or at least partial, equilibration occurred within the gut.

Results emanating from many studies show the wide diversity of water content in different species. Haines et al. (1974) stated that the normal mammalian range lay between 60 - 70% of body weight. In lean herbivores, water as a percentage of body weight is greater than in most other mammals because of the presence of large specialized fermentation zones containing water (Maloiy et al. 1979). Wright (1982) found that TBW in cattle was between 73,0 - 74,6% of total body weight. King (1982) considered 70% body water in African game animals to be quite acceptable as they have very little fat. In hartebeest, Alcelaphus buselaphus 84,3% of the body weight is water (Maloiy & Hopcraft 1971), and King (1979) found it to be 73% in eland.

King (1979) found oryx to have a TBW content of 73% ± 2,3%, while in another study King, Nyamora, Stanley-Price & Heath (1978) determined that 86,8% ± 0,92% of body weight was water in the oryx. Macfarlane & Howard (1972) found a body water content of 70% in the

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oryx. The present study found that the TBW ranged from 61% to 69,4% of body weight depending on the conditions to which the animals were exposed. These values were considered to be comparable to those obtained for other African herbivores.

The higher TBW recorded in summer as compared with winter, may be explained by Kamal's (1982) proposal that an increase in TBW is considered to be an adaptive response to counter the effects of heat stress. It is assumed that high water retention assists the animal in heat tolerance due to the high specific heat capacity of water. This slows down the elevation of body temperature. Excessive tissue hydration may explain the superior heat tolerance in camels and buffalo, Syncerus caffer, over Osemi sheep and Red Danish cattle. Camels and buffalo have body water contents of 213,2 l/100 kg body weight and 242,8 l/100 kg body weight respectively, while sheep and cattle have 186,9 and 161,6 l/100 kg body weight, respectively (Kamal 1982).

Another possible explanation for the increased TBW in summer is provided by Macfarlane, Howard & Siebert (1967) who found that TBW is greater when water turnover increases. This relationship though, is not absolute and was clarified by Macfarlane & Howard (1972) who stated that the general rule is "the more water that passes through an animal, the more that remains there in them". In the present study, a regression analysis of TBW against water turnover did not indicate any significant relationship between the two ($r^2=0,12$; p

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> 0,05) (data from Chapter 5). However it is believed that this is due to the small sample sizes involved. When mean values are plotted against each other, a trend appears to exist. This is seen in Fig. 9. where water turnover appears to decline as TBW declines.

The effects of dehydration on water dependent species are usually more severe than on those acclimated or adapted to water deprivation. Camels can tolerate a body water loss of 25% of their body weight compared to other animals where 15% is fatal (Etzion, Meyerstein & Yagil 1984). Dehydrated gemsbok suffered a net loss of 0,20 $1/kg^{0,82}$ of water in 5 days. This represented 2,7% of the body water content as a percentage of body weight at the end of the experiment. This is similar to the camel where Macfarlane & Siebert (1967) recorded a weight loss of 2% per day when given no water. In contrast, in baboons (Papio hamadryas), TBW declined by 12,5% after only 2 days (Zurovsky, Shkolnik & Ovadia 1984).

However the results of TBW determinations obtained by HTO during dehydration are subject to criticism since it can be argued that the animals were not in water balance. Nevertheless it is thought that the period of equilibration in TBW determinations will be too small to add any appreciable error to the determination. As regards water turnover calculations, which are considered in Chapter 5, it is believed that appreciable error would occur over the time period used for these determinations in dehydrated animals.

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The lack of significant differences in TBW recorded under the treatments applied indicates the ability of the gemsbok to regulate water loss. Moreover, the drinking of saline water in summer resulted on average in a greater retention of water than in dehydrated animals. The result for animals acclimated to saline water in winter may be explained by the suspected increase in renal excretion of electrolytes (see Chapter 3) with a concomitant increase in water loss. In the previous chapter some of the mechanisms involved were explained.

Intracellular fluid volume

In gemsbok, as in sheep, most of the fluid loss was from the ECFV. In contrast, camels lost more intracellular fluid (Macfarlane, Morris & Howard 1963).

In gemsbok, the stability of the ICFV indicates that it is regulated in the face of dehydration and saline water imbibition. Proper functioning of cellular metabolism is thereby assured.

In dehydrated animals ICFV was higher than values recorded in animals acclimated to fresh water in summer, while ECFV was significantly reduced. It would thus appear that ICFV does not support the ECFV, which may indicate that there is a mechanism to limit the loss of intracellular fluid.

Cellular water loss may increase with heat exposure (Horowitz &

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Samuelhof 1979). In the present study this was noted in summer in hydrated animals, but in dehydrated animals there was an increase in ICFV. This may have resulted from an increase in intracellular fat metabolism induced by a combination of decreased feed intake and water deprivation, as proposed by Reese & Haines (1978).

Erroneous measurement of TBW may also explain the increase in ICFV in dehydrated animals, since a reduction in ICFV during dehydration would have been expected as a result of the increase in the osmolality of the ECFV. TBW determinations may have been inaccurate in dehydrated animals which were not in water balance. Since ICFV was calculated from the subtraction of ECFV from TBW, any error in the TBW determination would propagate through to the ICFV.

Extracellular fluid volume

ECFV comprises 28,5% of body weight in cattle, 30% in sheep (Hix et al. 1959), and 22,1% in adult horses, Equus caballus, (Carlson, Harrold & Rumbaugh 1979).

The ECFV is regulated by the absorption of fluid from the intestine, and by volume sensors in the hepatic portal system and the atrium of the heart (Macfarlane & Howard 1972). It is also regulated by concentration sensors in the juxtaglomerular cells of the kidney working through angiotensin on the glomerular layer of the adrenal cortex (Macfarlane & Howard 1972). Since Na is the primary determinant of volume, the mechanisms that control Na

balance are the major mechanisms defending ECFV. Current opinion is that volume changes induce changes in ionic concentrations which through a readjustment in osmotic pressure produce changes in volume. Thus, excess Na in the extracellular fluid (due to some imbalance) may cause a shift in water from the cellular compartment into the ECFV (Gaur, Henry & Behn 1970), resulting in a new equilibrium.

This may be evident in hydrated gemsbok, where an increased plasma Na concentration in summer is accompanied by an increased ECFV. The increased electrolyte levels would have the effect of drawing in fluid from the intracellular fluid in an attempt to stabilize the osmotic increase in the ECFV. In winter, the ICFV is larger than the ECFV, and the plasma Na concentration is lower than that of summer (see Chapter 2).

Lavietes et al. (1936) also found that an increased ECFV may indicate an extreme degree of cellular wastage. Since the ICFV did not differ significantly between any treatments, it did not appear as though intracellular wastage occurred. However the higher ECFV in summer, also noted by Macfarlane et al. (1956) in young sheep, may have been supported by cellular water.

Dietary intake is also known to affect ECFV. When sheep were placed on a saltbush diet (high in Na and K) ECFV declined from 309 ml/kg to 210 ml/kg (Macfarlane et al. 1967). Conversely Hix, Evans &

Underburg (1953, in Macfarlane, Morris, Howard & Budtz-Olsen 1959) noted a 7,9% increase in ECFV to 33,2% of body weight in sheep given 21 g NaCl per day as opposed to 0,2 g per day.

In the present study it appears as though saline water has little effect on body water distribution in summer. However the reduction in ECFV and intracellular water in saline acclimated animals in winter may be the result of a suspected, if unsubstantiated, increase in the excretion of electrolytes and water (see Chapter 3), since TBW also declines.

ECFV is largely affected by the state of hydration of the animal. Macfarlane (1956, in Kutscher 1968) found that ECFV was reduced by 45% when Merino sheep were dehydrated. Hix et al. (1959) found that under conditions of mild dehydration ECFV declined from 30% to 28,5%, and further to 22,4% under severe dehydration.

Macfarlane & Siebert (1967) found that during rapid dehydration, ECFV loss occurred, but differentially between plasma and interstitial fluids, with interstitial fluid providing the major part. However Merino sheep, gerbils and laboratory rats lost relatively the same amount of fluid from the plasma fluid and interstitial fluid during dehydration (Reese & Haines 1978). In ruminants, Macfarlane & Howard (1972) reported that the interstitial fluid compartment was proportionately larger than in other animals, and could be drawn on when dehydration occurred.

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This is apparent in dehydrated gemsbok in Fig. 11., as plasma volume remained stable. This has important consequences for arid dwelling animals.

Plasma fluid volume

Plasma volume differs widely between species. Kami et al. (1984) found that in horses it comprised 4,7 - 6,3% of body weight, and Prothero (1980) found that there was a relationship between blood volume and body weight. His equation for the calculated blood volume of terrestrial male mammals was $0,081W^{0,99}$ and for plasma volume $0,046W^{1,0}$ (where W = mass in kg).

Gemsbok however, had plasma volumes that were on average larger than that predicted by the equation. Plasma volume in arid adapted species may be larger than in mesic species. Reese & Haines (1978) reported that plasma volume in Sinai goats is 8 - 10% of body weight. It would appear therefore, that arid dwelling animals have "extra" plasma in circulation. This could be advantageous when the animal is faced with water stress.

Kutscher (1968) noted that in men deprived of water there was a 6,5% loss of plasma volume for a 3% loss of body weight. Human beings subjected to heat stress and dehydration lost 2,0 - 4,4 times as much water from the plasma as from any other fluid compartment (Senay & Christensen 1965). In rats the situation is similar with a 28% loss of plasma volume for a 9,8% loss of body

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weight after 48 h of dehydration. Poor plasma conservers like the dog lose relatively more plasma than any other body fluid. This undoubtedly leads to fatality within days.

In animals considered to be arid adapted, plasma volume tends to be conserved under dehydration (Horowitz & Borut 1970; Horowitz & Samuelhoff 1979; Zurovsky et al. 1984). Denny & Dawson (1975) reported that camels lost only 10% of their plasma volume (relative to body weight) during a 20% loss of body weight. The advantage of conserving the plasma fluid would be to maintain cardiac output, arterial pressure and blood viscosity. In warm surroundings, loss of the ability to regulate these variables will result in circulatory stress. This will interfere with heat transfer (Zurovsky et al. 1984), since plasma fluid provides the initial supply of water for evaporation (Reese & Haines 1978).

Two mechanisms have been proposed to account for arid adapted species tending to lose less water from plasma than other compartments. The first involves a decrease in the permeability of the capillary wall to plasma protein, and the second, an increase in the production of plasma protein.

Horowitz & Borut (1975) stated that the decrease in the exchange of plasma protein between vascular and extravascular spaces is due to a decrease in the permeability of the capillary membrane to plasma proteins. The consequent increase in the retention of plasma

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proteins causes an increase in plasma oncotic pressure. The net effect is to retain fluid within the plasma due to an increased osmotic differential. Horowitz & Samuelhof (1979) demonstrated this mechanism by finding a 30,2% and 33,3% increase in colloid osmotic pressure in slight and severe dehydration, respectively, with a resulting shift of interstitial water into the vascular bed. However Senay & Christensen (1965) found no evidence for the preferential retention of albumin. They found that the increase in colloid osmotic pressure was due to increased protein production, which forms the basis of the second theory proposed.

In the present study, there was no significant evidence to confirm that plasma protein concentration was related to plasma volume (Fig. 12.). As plasma volume fell and plasma protein concentration rose, there was a decrease in total plasma protein, indicating that the increase in plasma protein concentration recorded was an artifact of the decreased plasma volume. In the case of dehydrated animals total plasma protein decreased from 47,1 g to 44,8 g, a 5,1% decrease. Plasma protein production was therefore not increased.

Horowitz & Borut (1970) also found that retention times of Evans Blue were increased in A. caharinus, a small rodent, during dehydration. Since Evans Blue binds almost exclusively to plasma albumin, this indicates an increased retention of plasma albumin. This was not found in the present study where the slopes of the

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disappearance curves of protein bound Evans Blue did not differ significantly from one another on any treatment. It therefore appears as though loss of plasma albumin from the blood fluid is similar for all treatments. This leads to the conclusion that the maintenance of plasma volume in water stressed animals cannot be explained by increased protein production nor by preferential retention of plasma albumin. Further investigation is necessary to determine the exact mechanism of plasma volume regulation during water stress.

In conclusion it is apparent that the body fluids are well regulated in saline water acclimated and dehydrated animals with the conservation of the plasma fluid and ICFV being of note. The decline in ISFV, as opposed to plasma fluid and ICFV, is considered to be an important adaptation in the arid dwelling gemsbok.

CHAPTER 5

FOOD, WATER AND DIGESTION

INTRODUCTION

Survival depends on obtaining enough food and water for the
maintenance of basic metabolism. Arid environments are known to be
harsh regions in this regard, since periods of drought result in
both a scarcity of food and limited water resources. Only animals
that can cope with these potential stressors will be successful
inhabitants of such regions.

Non-arid dwelling animals, such as the rat and rabbit, decrease
feed intake when faced with water stress (McKinley et al. 1983).
This would, no doubt, affect the intake of preformed water and
metabolic water gained from the food. However the camel, an arid
dwelling animal, maintains food intake under stress and
dehydration; a fact that Maloiy (1973b) considers to be one of the
most important adaptations to arid life in ruminants.

Environmental conditions, such as ambient temperature and saline
drinking water, will also cause changes in the water dynamics of
the animal (El Hadi & Hassan 1982). Without drinking water in
summer, animals such as buffalo, cattle, pigs (Sus scrofa), eland

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and waterbuck (<u>Kobus</u> <u>ellipsiprymnus</u>), will die within four to five days (Maloiy <u>et</u> <u>al.</u> 1979). Given 1% saline drinking water they lose co-ordination. The camel, however, can survive for 15 days without water and can tolerate higher saline concentrations.

Haines <u>et</u> <u>al.</u> (1974) pointed out that there were also genetic determinants of water turnover. They argued that since a genotype is subjected to selective pressures, the rate of water turnover might be lower in desert dwelling species than in those of tropical areas. This should be an adaptive response in desert dwelling species (Reese & Haines 1978). Indeed Youseff, Johnson, Bradley & Seif (1974) have noted that there tends to be a lower turnover of water in desert species compared with montane species.

Although faecal production is essential to remove waste products from the body, faeces contain water which may be needed during times of water stress. Clearly it would therefore be advantageous when water stressed, to either reduce the water content of the faeces or the faecal output. The extent to which this occurs in various species may be related to the ability to inhabit arid areas. Macfarlane (1964) found that water restricted cattle reduced their faecal water content to 60% of that in unstressed conditions, while sheep and camels reduced their faecal water loss to 50% and 45%, respectively. Furthermore, the donkey loses half the total water lost from its body through the faecal route at 22°C. However, this is reduced by 50% when the donkey is dehydrated under desert

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conditions (Maloiy 1970), indicating an ability to reduce faecal water content under stress.

The KGNP is a region not only associated with periodic droughts, but also characterized by the provision of saline drinking water. In times of drought, or when water is required, animals inhabiting this region are faced with the prospect of either not drinking, or drinking saline water. The effects of this on their food intake, water turnover and other associated variables is not known, and clearly requires investigation.

MATERIALS AND METHODS

The basic experimental protocol is described in Chapter 1.

Food intake and apparent dry matter digestibility
Food intake and faecal output were measured on a dry weight basis. Aliquots of food and all faecal material produced were dried to constant weight in an oven at 60°C. Data from these determinations were used to convert wet weights to dry matter intake and dry matter faecal output.

Digestibility trials were carried out over five days. On the day preceding the trial (Day 14 of the experimental protocol in Hydration trials and Day -1 for Dehydration trials), all food was withdrawn. Food was supplied the following morning and the faecal

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material produced in the first day was discarded. It was assumed that "todays faeces was yesterday's food". Faecal collection began on Day 2 of the digestibility trial, and was terminated on Day 6. Apparent dry matter digestibility was calculated from the following equation -

$$\text{\% Apparent dry matter digestibility} = \frac{\text{dry matter intake} - \text{dry faecal output}}{\text{dry matter intake}}$$

Energy

Food and faecal samples were collected, dried at 60°C to constant weight, and their energy contents determined by bomb calorimetry. Total energy intake was calculated by multiplication of the dry matter intake by the energy content of the food. Total faecal energy content was calculated by multiplying dry mass faecal output by the energy content of the faeces. Digestible energy (DE) was determined from the following equation -

DE = Total energy intake - Total faecal energy output

The efficiency of energy extraction from the food eaten was calculated by dividing the energy extracted (total energy intake minus total faecal energy output) by the total energy intake and expressing this as a percentage.

Faecal water content

Faecal water content was determined as g H_2O/100 g dry faeces,

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after weighing, and drying the faeces at 100°C to constant mass.

Water intake

Drinking water intakes were measured by subtraction of the water remaining in the water trough from the measured amount provided. Evaporation was corrected for. Preformed water was measured by determining the change in mass of an aliquot of food after drying at 100°C to constant weight. Metabolic water was calculated after determining the protein, fat, carbohydrate and ash content in the food.

Crude protein content was determined using the Kjeldahl method (Horowitz 1980). In this determination, it was assumed that all food protein contains 16% nitrogen, and that all the nitrogen present was contained in protein. It was also assumed that all protein was digestible.

Fat content was determined following the "direct method" of Horowitz (1980).

The ash content of the food was determined by placing a measured amount of food in a furnace at 600°C, and determining the mass of the original food sample remaining after heating. The carbohydrate content was then calculated by subtraction of the combined % protein/fat/ash contents from 100.

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Metabolic water production/g dry food was calculated using the values for metabolic water production given by Schmidt-Nielsen (1983). The metabolic water produced from the food digested was calculated by subtracting dry faecal output from dry food intake, and multiplying this value by the metabolic water content of the food.

Water turnover

Water turnover was measured in hydrated animals using HTO. Determinations of turnover rate in dehydrated animals were initially performed, but did not prove to be accurate as explained in Chapter 4, and were therefore discontinued.

HTO was introduced into the animals using the method described in Chapter 4. The radioactivities of the equilibration sample drawn on Day 1 of the experimental phase, and a blood sample drawn on Day 5 of the same phase, were determined by scintillation counting as described in Chapter 4. These values were converted to their natural logarithms. The time difference between the drawing of the two samples was also determined. This enabled calculation of the decrease in HTO concentration with respect to time, and hence to the fraction of HTO space turned-over per day (k) -

$$k = \frac{\ln \text{(Equilibration sample dpm)} - \ln \text{(Last sample dpm)}}{\text{Time from Equilibration sample to Last sample}}$$

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The time required for the activity of the isotope to decline to 50% of the original activity is known as the biological half life, or $t_{1/2}$ (Hudson & Rummel 1966). $T_{1/2}$ was calculated from the following relationship -

$$t_{1/2} = \frac{\log 2}{a}$$

where - a = slope of regression line joining dpm
of equilibration sample with that of
last sample.

RESULTS

Food intake

Food intake in summer declined when gemsbok were acclimated to saline drinking water, compared with those acclimated to fresh water. However in winter, food intake in gemsbok acclimated to fresh water was similar to that in summer, while it increased in animals acclimated to saline water. Since none of these results were significantly different from one another, it appears that the water treatments in both seasons had little effect on food intakes. However there was a significant decline of 51% in food intake when animals were dehydrated (Fig. 13.), as compared with animals acclimated to fresh water in summer.

Faecal output

Faecal output was greater in winter than in summer for both water treatments (Fig. 13.), although the only values that differed

93
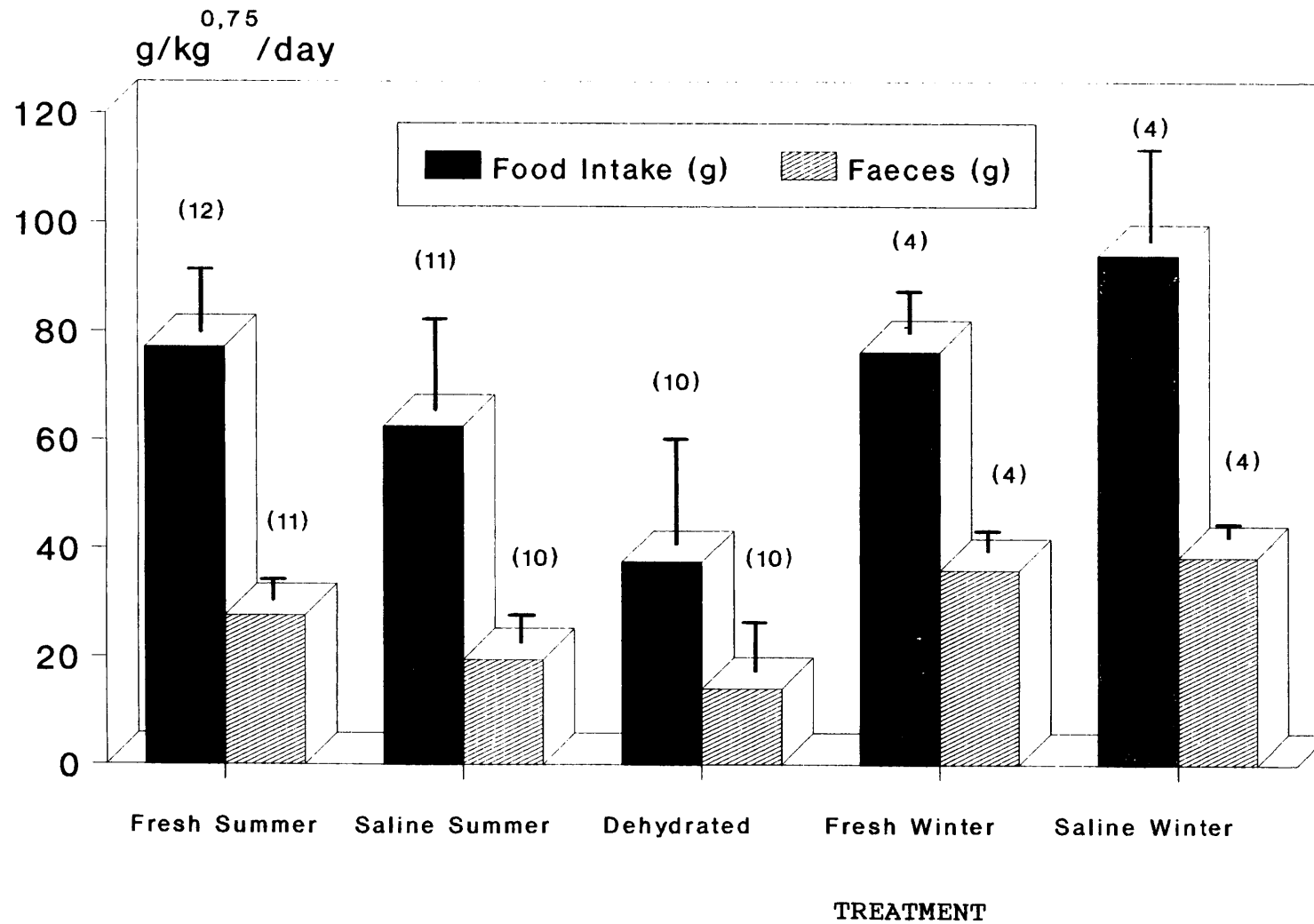
Fig. 13. Food intake and faecal output in gemsbok exposed to different treatments. Results presented as means + 1 S.D. Sample size in brackets.

significantly were those for animals acclimated to saline water in summer and those acclimated to saline water in winter. Dehydrated animals defaecated the smallest amount of faeces, and this was significantly different from that defaecated by animals acclimated to fresh water in summer.

Apparent dry matter digestibility

Figure 14. shows that the percentage dry matter digestibility increased in both summer and winter when the animals were acclimated to saline water, although it was lower in winter than in summer on all treatments. Dehydrated animals had similar dry matter digestibilities to those found in animals acclimated to fresh water in summer. No significant differences were found at the 5% confidence interval, although at the 10% confidence interval, saline acclimated and dehydrated animals had significantly different digestibilities from animals acclimated to fresh water in winter.

Energy

The highest DE was found in saline acclimated animals in winter (35394 $\pm$ 13953 kJ/day, n = 4). Animals acclimated to fresh water in winter had a DE of 27474 $\pm$ 6334 kJ/day (n = 4), and those acclimated to fresh and saline water in summer had DE intakes of 31926 $\pm$ 6936 kJ/day (n = 11) and 24881 $\pm$ 5977 kJ/day (n = 10), respectively. Dehydrated animals were found to have a significantly different DE value from all other treatments (10216 $\pm$ 3255 kJ/day,

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n = 6). The results for the percentage energy assimilated are shown in Fig. 14. Animals removed more energy from the food eaten in summer than in winter. Surprisingly low values of energy assimilation were recorded in winter on both water treatments. Although a higher energy assimilation was found in dehydrated gemsbok, this was not significantly different from other treatments.

Faecal water

In summer the water content of the faeces declined significantly from 152,98 ± 34,93 g H_2O/100 g dry faeces to 87,23 ± 19,50 g H_2O/100 g dry faeces in fresh (n = 11) and saline (n = 11) water acclimated animals, respectively. There were also significant differences between fresh (n = 4) and saline water (n = 4) acclimated animals in winter (151,40 ± 39,00 and 88,82 ± 53,24 g H_2O/100 g dry faeces, respectively). Significant differences were also found between dehydrated animals (48,90 ± 14,01 g H_2O/100 g dry faeces, n = 10) and those acclimated to fresh water in summer.

Water intake

Water intake was significantly lower in saline water acclimated animals than in fresh water acclimated animals in summer. Winter water intakes were comparable on either treatment. Figure 15. shows the contributions made by metabolic, preformed and drinking waters to total water intake.

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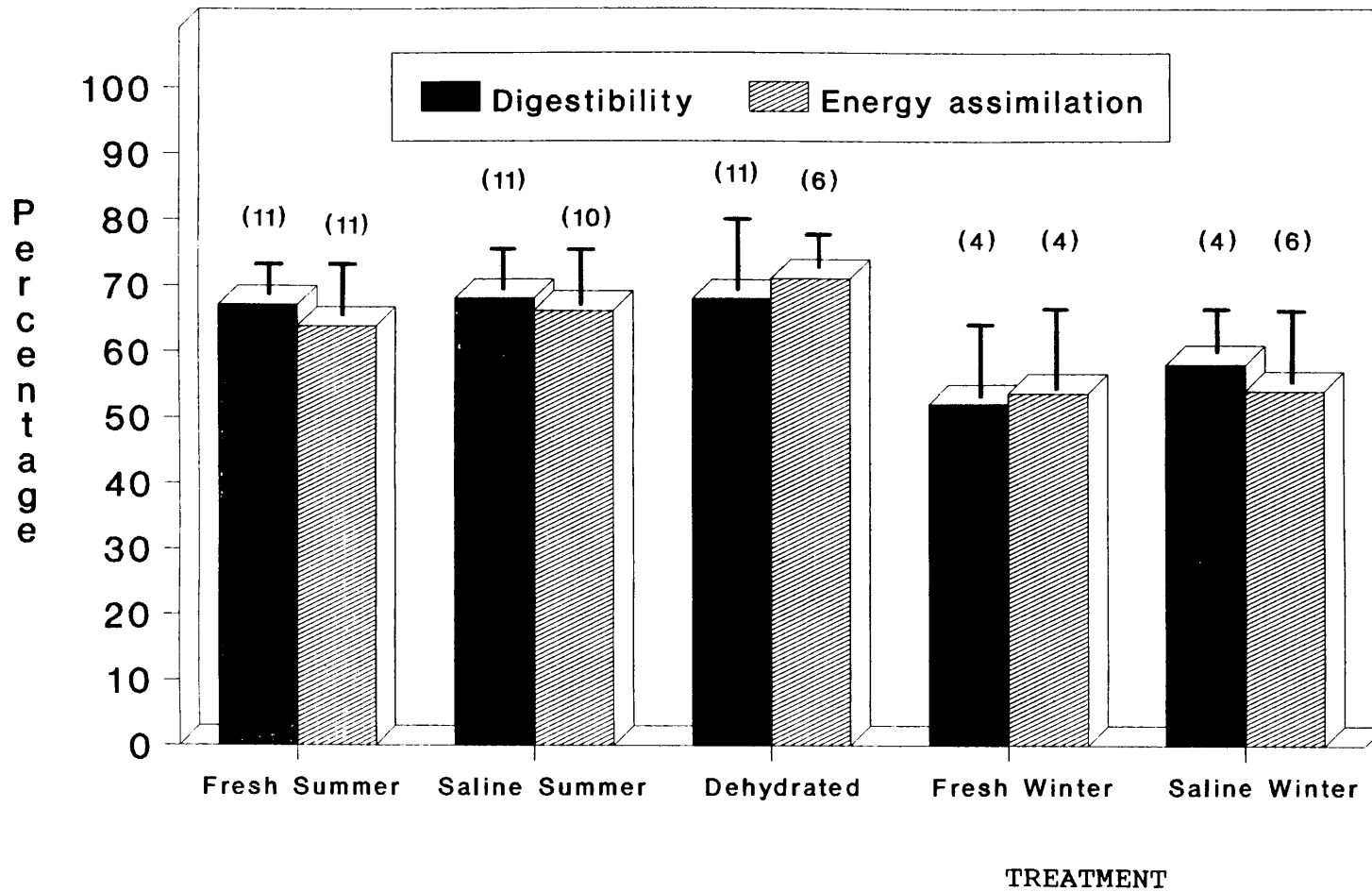
Fig. 14. Apparent dry matter digestibility and energy
assimilation in gemsbok exposed to different treatments.
Results presented as means + 1 S.D. Sample size in
brackets
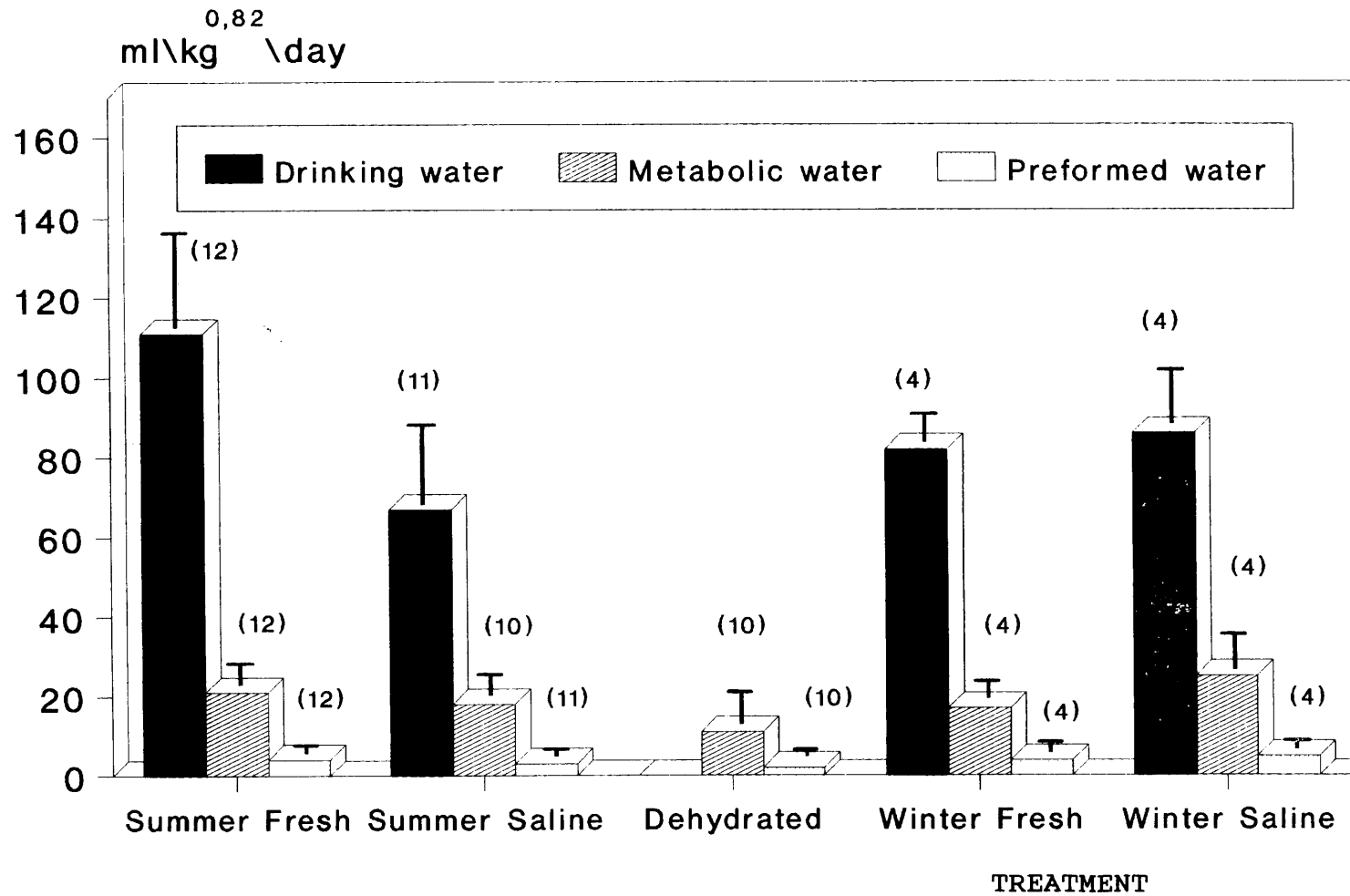
Fig. 15. Drinking water, metabolic water and preformed water contributions to total water intake in gemsbok. Results presented as means + 1 S.D. Sample size in

Water turnover

The average water turnover in summer was 149,69 $\pm$ 51,6 and 115,05 $\pm$ 23,03 ml/kg^{0,82}/day for animals acclimated to fresh (n = 6) and saline (n = 4) water, respectively. In winter, turnover rates were 103,29 $\pm$ 42,29 and 85,99 $\pm$ 20,90 ml/kg^{0,82}/day for fresh (n = 2) and saline (n = 2) water acclimated animals, respectively. Significant differences were not recorded, although trends could be distinguished.

The biological half life of HTO in fresh water and saline water acclimated gemsbok was 8,3 and 9,1 days, respectively, in summer and 12,3 and 13,0 days, respectively, in winter. Summer and winter values differed significantly, but there was no significant intra-seasonal difference between water treatments.

DISCUSSION AND CONCLUSIONS

Food, faeces, digestibility and energy

The need to maintain food consumption at a level which will satisfy the minimum energy and nutritional needs was considered by Haines, Cisowski & Harms (1973) to be an important factor in acclimation to water restriction. In mice that had been completely acclimated to water restriction, food intake was similar to that for mice on ad lib. water (Haines et al. 1973).

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This was not found in sheep, where, after two days of water deprivation, food consumption ceased (Macfarlane et al. 1961). Furthermore, laboratory sheep fed lucerne were not able to tolerate water deprivation at 40°C for more than three days (Macfarlane et al. 1961). In hydrated oryx, average daily food intake at 22°C was 2,23 kg/100kg bd. wt/day, and when dehydrated, 1,33 kg/100kg bd. wt/day. Under simulated desert conditions it was 2,25 kg/100kg bd. wt/day in hydrated animals and 1,76 kg/100kg bd. wt/day in dehydrated animals (Maloiy 1973b). Mohamed et al. (1988) found that when dehydrated, Dorcas gazelle reduced food intake by 41,8%. The reduction in food intake was also noted in dehydrated hartebeest (-15%) and impala (-23%) at 22°C. Under desert conditions, both species reduced food intake by 24% (Maloiy & Hopcraft 1971). In the present study, gemsbok decreased food intake by over 48% when dehydrated.

Although dehydrated gemsbok did not stop eating, and were able to tolerate dehydration for 5 days without fatality, it appeared that they were by no means acclimated to water deprivation. However, a reduced food intake would be advantageous since the intake of electrolytes and osmotically active substances would decline. The need to expend water in the excretion of these substances would therefore be reduced. That this occurs is well known in animals exposed to water deprivation (Thrasher et al. 1984). However, the reduction in food intake would also lead to a decreased energy intake.

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The 5% increase in the apparent dry matter digestibility and the 7,26% increase in energy assimilation found in dehydrated gemsbok may be instrumental in reducing the energy and nutrient debt incurred as a result of a reduced food intake. Maloiy (1970) also reported an increase in the dry matter digestibility in water deprived donkeys. In the latter species, complete water restriction at 22°C depressed food intake by over 80% at the end of 8 - 12 days. At a 15% level of dehydration, food consumption declined by 30,4%, but apparent dry matter digestibility increased from 41% to 51%.

The observation that, in gemsbok, dry matter digestibility was reduced in winter for both water treatments, was at first inexplicable. However literature values for apparent dry matter digestibilities in other species are in the range of that determined for gemsbok in winter. It therefore appears that values recorded in summer are high. This may indicate an important water conserving adaptation. Taylor, Spinage & Lyman (1969) suggested that the increase in digestibility found in dehydrated waterbuck (62% to 69%) could account for half the reduction in faecal water loss, the other half coming from the reduction in faecal water loss when water was restricted.

Saline water is also known to affect food intake. Wilson (1966a) found that food intake declined on 1,2% NaCl and Pierce (1957a,b in Wilson 1966a) found decreased food intake in sheep as a result of

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drinking saline water. Wilson (1966b) quoted Weeth & Haverland (1961) as finding decreased food intakes in cattle ingesting saline drinking water, an observation also noted by Wilson (1966a).

In sheep, digestibility was not seriously affected when saline drinking water was provided (Wilson 1966b). However a decrease in digestibility, due to increased passage of food through the gut, has been reported (Elam 1961, in Wilson 1966a). In the present study, gemsbok given saline water in summer had higher digestibility and energy assimilation percentages than did those given fresh water. Moreover, the apparent digestibilities of saline water acclimated and dehydrated animals were significantly different ($p < 0,1$) from those of fresh water acclimated animals in winter, implying that heat and water stress may influence digestibility. Consequently, as a result of the increased digestibilities it can be concluded that less food need be consumed to obtain the same amount of nutrients and energy. This is important in summer when water is scarce. Stanley-Price (1985) found that while fringe eared oryx (<u>Oryx</u> <u>beisa</u> <u>callotis</u>), ate a similar quantity of food to that of sheep, but less than cattle, all three extracted the same proportion of nutrients. This is an advantage in reducing food intake while obtaining the necessary nutrient input.

However, it is difficult to explain why animals with <u>ad</u> <u>lib.</u> fresh water in summer should have increased digestibility ratios, unless

there is some effect of heat on their system.

Percentage energy assimilation is considered to be a more useful indication of energy status change than is DE. This is explained by the fact that DE does not account for the amount of food eaten to provide that energy. Hence, it does not describe the efficiency of energy extraction. It is evident that, although dehydrated gemsbok reduce food intake and hence energy intake, there is an increase in the amount of energy extracted from the food eaten compared with hydrated gemsbok. The advantage of this, with regard to water economy, has been explained above. A similar increase in energy assimilation was found in saline water acclimated animals between summer and winter. The same advantage applies here too. The low values obtained in winter may be explained by the observation that it is believed that water is not as limiting a factor in winter as it is in summer (see Chapter 3). As such, in winter, faecal water loss is not considered a major problem and both fresh and saline water acclimated animals could eat more food.

Digestibility and energy assimilation are reduced in winter, and this may result from reduced heat and water stress in winter. Yet, since within any season, saline water is considered more stressful, increased values are associated with it. There was a significant correlation between energy assimilation and digestibility in all animals ($r^2 = 0,67$; $p < 0,05$).

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Faecal water loss is related to the amount of food eaten, the digestibility and the water content of the faeces. As such, the more completely the food is digested, the greater the reduction in faecal water loss (Taylor et al. 1969). In the present study this was only evident within seasons where decreased faecal water loss was found in animals with increased digestibilities. Between seasons, animals on fresh water had similar faecal water losses as did those on saline water. It is therefore apparent that water quality and not season resulted in changes to the faecal water loss.

However, Maloiy & Hopcraft (1971) found in hartebeest and impala that not only was there a decrease in faecal water loss when the animals were water restricted, but also when temperatures were increased. No evidence for temperature effects was found in the present study, although the results are in agreement with Maloiy & Hopcraft (1971) that the decrease in faecal water content was greatest when animals were water restricted.

Donaldson & Edwards (1981) found that faecal water content declined to 13% of control values in dehydrated animals, and this was attributed not only to a reduction in faecal water content, but also to reduced faecal output. This was also found by Maloiy & Taylor (1971) who found that water restriction resulted in a 61% reduction in faecal water loss in the Turkana goat, and a 57% decrease in the fat tailed sheep. Half of this loss was attributed

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to drier faeces and the other half to reduced food intake.
MacMillen & Lee (1969) also found reduced food intake in water
deprived rodents, and this resulted in a 33% reduction in faecal
production.

Macfarlane & Howard (1972) attributed part of the reduced faecal
water loss to some colon sited water absorption mechanism, while
Maloiy (1970) suggested that the reduction in faecal water content
was due either to larger solute linked water flow in the large
intestine and rectum, or to the release of a mineral corticoid that
increased intestinal Na reabsorption.

The latter suggestion could explain why Donaldson & Edwards (1981)
found that in saline treated gerbils, there was an increase in
faecal water loss. Given saline water, intestinal Na uptake would
tend to be reduced as a result of a decrease in the release of the
mineral corticoid. The nett result would be an increased faecal
water content. Another possible explanation for the increased
faecal water content relates to the reduced osmotic movement of
water out of the intestine, since the presence of dissolved
electrolytes in the intestine reduces the water gradient.

In gemsbok, the observation that saline water acclimated animals
had a reduced faecal water loss may be indicative of the fact that
Na linked water uptake in the intestine was not reduced. This may
be further supported by examining kidney function and plasma

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electrolyte concentrations. In winter on saline water, an increased GFR increased the Na filtered load, which may have removed excess Na associated with the solute linked transport. Plasma levels of Na remained constant. However in animals given saline water in summer, the GFR was reduced, which reduced the filtration of Na ions, and the plasma Na concentration increased.

Water

Total water intake in fresh water acclimated animals in summer was higher than that of fresh water acclimated animals in winter. This is probably a result of the increased heat load in summer, which would increase the evaporative water loss, and necessitate increased water intake. Saline water acclimated animals in summer had reduced water intakes compared to fresh water acclimated animals, but in winter, saline and fresh water acclimated animals had similar total water intakes. This is indicative of the shortfall in water requirements in saline acclimated animals in summer, and explains why their physiology was so different.

The differences in drinking water intakes of animals in winter and summer is indicative of changes in water requirements due to heat. Macfarlane et al. (1956) noted that in tropical Merino sheep in winter, mean water intake was 7,3 ml/kg/day while in mid-summer the intake increased to 88,8 ml/kg/day. King et al. (1978) found that the total water intake averaged 28,12 ml/kg/day in 1,5 year old oryx. Taylor (1968) found that at 22°C, oryx needed 5,66

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$1/100kg^{0,75}/day$ (1,88 $1/100kg/day$) or 9,04 $1/100kg^{0,75}/day$ (3,00 $1/100kg/day$) under simulated desert conditions. Maloiy (1972) found that in the camel, 2,06 $1/100kg/day$ was required at 22°C, or 3,84 $1/100kg/day$ was needed under simulated desert conditions. Water intake in sheep was also considerably greater than in oryx and camels as they required 6,81 $1/100kg/day$ (Purohit, Ghosh & Taneja 1972).

Determination of the minimum water requirements in gemsbok was not possible for reasons previously stated (Chapter 1). However when dehydrated, gemsbok lost weight at approximately 2 - 3% per day. Given a diet consisting solely of lucerne, containing 6 - 8% preformed water, survival without drinking water would be impossible. Condition estimation based on weight loss, flaccid skin, sunken eyes, and refusal of food indicated that, after a loss of 15% of body weight, animals were in poor condition. Based on the assumption that a 25% loss of body weight would be fatal, it is believed that 10 days would be the maximum time period a gemsbok could be exposed to these conditions without fatality.

However, there is a need to extrapolate these results to natural conditions, and although this can never be exact, a rough estimate of the minimum water needs can be made. It is assumed that all other factors remain constant in captive and wild gemsbok. If it is assumed that natural grasses contain 20% preformed water, that there is a 60% digestibility, and the metabolic water production

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amounts to 0,5 g H_2O/g food, then to obtain the amount of water used by a 120 kg dehydrated captive gemsbok (608 ml H_2O/day), approximately 1,3 kg dry weight of food needs to be eaten. From feeding records, up to 3 kg dry weight food can be eaten per day. Under natural conditions this corresponds to approximately 1,5 l H_2O/day. This would provide an additional 0,9 l H_2O/day to that obtained by captive animals. From records of dehydrating captive animals, there is a 2% loss of body mass per day, corresponding to 2,4 kg/day in a 120 kg animal. With the additional water gained by a wild living animal, this loss of body mass would be reduced to 0,9 kg/day, or less than 1% of body mass per day. Survival time would be extended from 10 to 25 days. There are two assumptions in this calculation - first, that only water was lost from the body, and second, that a dehydrating animal would eat normally. Since it is conceivable that some body solids would be lost, the water debt would not be as great as that calculated. However this is likely to be countered by a reduction in food intake in dehydrating animals. Should the water content of the food eaten increase, the animal could become totally independent of water.

Gemsbok reduced saline water intakes in summer. This is believed to be due to the inability of the kidney to excrete the electrolytes (See Chapter 3). Yet, in winter, saline water intake was greater than that of animals acclimated to fresh water. The camel was also found to increase water intake on saline water from 2,93 l/day to 5 - 12 l/day (Maloiy 1972). It is believed that, in gemsbok,

changes in renal function are involved in the excretion of greater electrolyte loads in winter, and hence more water can be drunk. Furthermore, it is thought that saline water of the concentration used in the present study is barely tolerable in summer. Sheep are known to tolerate 1,3% NaCl in their drinking water while cattle only tolerate solutions up to 1% (Macfarlane & Howard 1972). Macmillen & Lee (1969) found that the drinking of saline water was directly proportional to the concentration of salt in the water, and it is believed that a salinity of this nature would enable sustained drinking of saline water in gemsbok.

Nicol (1978) gave the water turnover rate for mammals as $102,2b^{0,82}$ (where b is in kg). According to this equation, water turnover in gemsbok averages 5,2 l/day, or 102 $ml/kg^{0,82}$/day. However differing environmental and physiological conditions would tend to limit the use of this equation. The mineral composition of the food and water will also influence the water turnover (Hume 1982). Gemsbok, for example, decreased water turnover when acclimated to saline drinking water in both summer and winter.

In the present study, the turnover of water in summer increased, probably in response to an increased heat load. Season has been shown to affect water turnover rate. Ranjhan, Kalanidhi, Gosh, Singh & Saxena (1982) found that in sheep, goats and crossbred cattle, turnover was higher in summer than in winter. This was also found in the camel (Macfarlane & Siebert 1967). Kamal (1982)

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reported that Osemi sheep had an increased turnover in summer coupled with an increased TBW.

Water turnover in gemsbok was higher than that recorded in camels (3,91 l/100kg/day in camels (Kamal 1982), equivalent to 98 ml/kg^{0,82}/day in gemsbok) but was lower than that of non-arid dwelling species. Kamal (1982) reported that water turnover in sheep was 10,07 l/100kg/day, cattle 7,52 l/100kg/day and buffalo 9,6 l/100kg/day. In the hartebeest, Maloiy & Hopcraft (1971) found that 116 ml H$_2$O/kg^{0,82}/day were turned over as opposed to cattle where over 222 ml H$_2$O/kg^{0,82}/day were turned over. Cattle thus turned over 90% more water per day. Macfarlane, Howard, Haines, Kennedy & Sharp (1971) found, however, that oryx used water at a slower rate than camels, and Maloiy (1973b) found that at 70 ml/kg^{0,82}/day the oryx used less water than any other animal studied. The present study showed substantially increased turnover rates from those of the East African oryx.

Decreased water turnover results from water deprivation (Haines et al. 1973). This has also been reported by Hewitt et al. (1981) in the desert rodent, Notomys alexis, and by Haines et al. (1974) in dassyurids and murids. In the present study, it is likely that there was a reduction in water turnover in dehydrated animals. This is brought about, in the main, by reducing metabolism and minimizing renal water loss (Etzion et al. 1984). Taylor (1969) found that when oryx were dehydrated, they lost little water from

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the skin and panted instead. However when hydrated 75% of the total water lost was lost through the skin.

Naturally the amount and type of food eaten will affect the water relations within the body. Siebert (1971, in Macfarlane & Howard 1972) working on tropical cattle, obtained a correlation of 0,84 between food intake and water turnover. One of the reasons that water turnover is related to food type and consumption, is because of the energy released, and Macfarlane et al. (1971) stated that there is a relationship between energy and water turnover. In the present study, significant correlations existed between water turnover and food intake (r^2=0,53 ; p<0,02) and water turnover and energy assimilation (r^2=0,46 ; p<0,04) in both water treatments, but only in summer. It is believed that the energy status of the body (and hence food digested), will affect the water relations since evaporative cooling may be required (King 1982; Finch & King 1982). In gemsbok, it is thought that during winter, increased feeding is not linked to increased water turnover because the extra heat generated does not require dissipation. It is, in fact, needed. This may be seen to provide further evidence of the effect of heat on the water physiology of the gemsbok, with winter providing some "elasticity" on the link between food consumption and water turnover.

The biological half life is related to the water turnover rate, in that the faster the turnover rate, the less the biological half

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life. Thus, in dehydration when water turnover rates generally decline, biological half life values increase. Hudson & Rummel (1966) found the half life increased from 4 - 9 days during dehydration in the heteromyids, Liomys salvari and L. irroratus. A similar trend was found by Black, Baker, Bartley, Chapman & Phillips (1964, in Wright 1982).

In the present study the effects of season are clearly illustrated. Naturally any factor increasing water turnover will decrease the biological half life, and the effects of season are well noted. Springell (1968) found that the biological half life of HTO was lower in warmer months, as did Wright (1982) who found that the half life of water in cattle increased from 78,2 h in autumn to 170,8 h in winter. It then declined in summer to 74,4 h. Ranjhan et al. (1982) found that the half life decreased from winter to summer in sheep (220 h to 98 h), buffalo (112,3 h to 75,3 h), crossbred cattle (102,6 h to 64,3 h), crossbred sheep (97,6 h to 15,6 h) and goats (134,3 h to 85,0 h).

To conclude, it is apparent that seasonality affects food intake, digestibility and water metabolism more than the provision of saline drinking water. Estimated minimum water requirements appear to be met in the natural state by the water obtained in the food, although drought would necessitate the provision of suitable water if survival is to be ensured.

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SUMMARY

The distribution of the gemsbok is largely confined to the arid regions of Southern Africa, where surface water may be limited or saline in nature. Failure to obtain adequate water from the diet may lead to dehydration and/or imbibition of saline water. The present study was aimed at determining some of the effects of dehydration and the intake of saline water on the physiology of the gemsbok.

Fewer significant changes in the variables measured were found between fresh water and saline water (1,8%) acclimated animals, than between hydrated and dehydrated animals. It was concluded that the effects of dehydration were more stressful than those resulting from the imbibition of saline water.

The regulation of plasma fluid volume and the intracellular fluid volume, at the expense of the interstitial fluid, was found to be an important determinant of survival in dehydrated animals. Similarly, the regulation of blood variables ensured the continued functioning of the circulatory system.

Dehydration, and to a lesser extent, the imbibition of saline water, resulted in a non-significant decline in the body water pool. Water turnover also declined when saline water was imbibed, indicating that water conserving mechanisms were operative.

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Renal function was significantly altered by dehydration, whereas imbibition of saline water had less effect. The trends recorded indicated that the kidney was responsible for the excretion of excess electrolytes and the conservation of water.

Another important water conserving mechanism recorded in dehydrated animals involved the increase in energy assimilation from food eaten. In addition faecal water loss was reduced in dehydrated gemsbok and to a lesser extent in gemsbok that had imbibed saline water.

Seasonal differences were apparent in many of the variables measured. With regard to water metabolism, summer appeared to be more stressful than winter.

It is concluded that gemsbok show adaptations to life in a region where periods of dehydration and saline water imbibition are common.

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OPSOMMING

Die verspreiding van die gemsbok is grootliks beperk tot die dorre streke van Suidelike Afrika, waar oppervlakwater skaars of sout van geaardheid mag wees. Die onvermoë om voldoende water uit die dieët te verkry mag tot dehidrasie en/of inname van soutwater lei. Die doel van hierdie studie was om sommige gevolge van dehidrasie en die inname van soutwater op die· fisiologie van die gemsbok te bepaal.

Minder beduidende veranderings is gevind vir die gemete parameters tussen gehidreerde en gedehidreerde diere as tussen varswater en soutwater geakklimatiseerde diere. Die gevolgtrekking kan gemaak word dat die effek van dehidrasie meer spanning veroorsaak as wat die geval is by inname van soutwater.

Daar is gevind dat die regulering van plasmavloeistofvolume en intrasellulêre vloeistofvolume, ten koste van interstisiële vloeistof 'n belangrike faktor vir die oorlewing van gedehidreerde diere is. Net so verseker die regulering van bloed parameters die funksionering van die bloedsisteem.

Dehidrasie en, tot 'n mindere mate die inname van soutwater, het tot 'n nie-beduidende afname in die liggaamswatervolume gelei. Wateromset het ook afgeneem tydens die inname van soutwater, wat 'n aanduiding van 'n aktiewe waterbesparingsmeganisme is.

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Nierfunksie is betekenisvol beïnvloed deur dehidrasie, terwyl soutwater inname 'n kleiner effek gehad het. Die waargenome neigings dui aan dat die nier verantwoordelik is vir die uitskeiding van oortollige elektroliete en die besparing van water.

'n Verdere belangrike waterbesparingsmeganisme wat waargeneem kon word by gedehidreerde diere was die verhoogde energie-assimilasie van voedsel wat ingeneem is. Voorts was daar ook 'n afname in waterverlies deur ontlasting by 'n gedehidreerde gemsbok, asook, tot 'n mate, by 'n gemsbok wat soutwater ingeneem het.

Seisoenale verskille was duidelik sigbaar in heelwat van die gemete parameters. Wat watermetabolisme betref, het die somer meer stres veroorsaak as wat die geval is in die winter.

Die gevolgtrekking wat gemaak word is dat die gemsbok aanpassings toon vir lewe in 'n gebied waar periodes van dehidrasie en die inname van soutwater 'n algemene verskynsel is.

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