THE HANDLING OF CONSTANT VOLUMES OF VARIOUS CONCENTRATIONS OF SEAWATER BY THE JACKASS PENGUIN *SPHENISCUS DEMERSUS*

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ABSTRACT

This paper reports on the effects of varying the concentration of seawater dosed at a rate of 10% of body mass on the handling of fluid and solutes by jackass penguins (*Spheniscus demersus*). The salt gland only secreted when the seawater dosed was hypertonic to the plasma and caused a rise in plasma osmotic concentration of at least 7,5%. The gland reacts to different marginal osmotic loads (equivalent to 40 and 50% seawater) by varying the flow rate and total concentration to match the degree of osmotic stress. High osmotic loads resulted in maximum flow rates and concentration from the onset of secretion. The relative composition of the ions was not affected by flow rate. Cloacal excretion decreased with a decreasing osmotic load, especially at those seawater concentrations that failed to stimulate the salt gland. This cloacal control is interpreted as a graded mechanism to dispose of osmotic loads. The cloacal system is stimulated to excrete by the same stimuli as the salt gland. Apparent selective Na^+ absorption by the cloaca was also noted.

INTRODUCTION

In a previous article (Erasmus in press) I investigated the elimination of solutes in jackass penguins dosed with seawater at 10% of their body mass. This solute load was calculated to mimic the maximum osmotic stress these birds would have to cope with when feeding exclusively on invertebrates. The jackass penguin may, however, also take fish or a combination of fish and invertebrates (Rand 1960 and personal observation) which would impose less osmotic stress on the bird. Two questions then arise. What osmotic load is needed to elicit a salt gland response, and how are the various osmotic loads handled by the different excretory organs of the bird? These problems also relate to results (Erasmus in press) from which I concluded that the digestive tract, including the cloaca, is an important osmoregulatory organ. The importance of the digestive tract, however, depends to some extent on the high initial flow rates of the cloacal fluid after dosing with seawater. Although I presented data to indicate that these high initial flow rates should be viewed as a physiological mechanism for excreting solute loads, Douglas (1970) interpreted high cloacal excretion rates in herring gulls as evidence of slight catharsis and therefore presumably as an artefact of the experimental procedure. It may also be argued that these high flow rates are only the result of mechanical fill.

The present experiment therefore reports on the effect of keeping the volume of fluid used in oral-loading the penguins constant at 10% of body mass while varying the concentration

of the seawater dosed. The handling of solute loads by penguins was also investigated. The volume loads were kept constant to eliminate any possible volume effects on flow rates or solute handling.

METHODS

The experiment was conducted on St Croix Island off the south-east coast of South Africa, where 20 penguins were caught at random from birds preparing to breed. Care was taken to exclude premoult or moulting birds to prevent the possibility of working with birds of varying degrees of dehydration (Douglas & Neely 1969). In a preliminary investigation the plasma composition of nine of these prebreeding birds was compared with that from eight other prebreeding birds immediately upon their return from a feeding trip from the sea. There were no statistically significant differences in plasma Na^+, K^+, Cl^-, osmotic concentration or albumen content between these groups and it was concluded that these prebreeding birds represent birds in their natural state with salt glands unaffected by compensatory hyper- or hypotrophy (Peaker & Linzell 1975).

The 20 penguins were weighed and orally loaded with different concentrations of seawater all made up from 100% seawater (SW) with distilled water to a volume equal to 10% of the mass of each bird. The seawater had the following analysis: 1080 mOsm/l; $Na^+ = 468$; $K^+ = 10{,}1$ and $Cl^- = 546$ meq/l. Five birds were used at 100% SW but only two or three per treatment at 75%; 50%; 40%; 35%; 25% and 10% SW. For the purpose of this experiment it was regarded as preferable to use many seawater concentrations but fewer replicates at each concentration. The dosing, collection of excretions and analysis of the composition of the fluids collected were the same as by Erasmus (in press).

Collections were made at 30 minute intervals for 120 minutes after dosing. In addition, plasma samples were taken from some birds at each treatment during and at the end of the collection period.

RESULTS

The 20 penguins used had a mean mass of 3680 ± 44 g.

Salt gland excretion

Salt gland secretion only occurred when the seawater concentration was 40% or more. The onset of secretion was retarded at less than 100% SW loads (Figure 1), and the lag in time for the first secretion to occur increased with decreasing osmotic load. The secretion rate was not maximal from the start and needed more than 30 minutes to attain peak flow during all the treatments. Maximum flow rate varied between 0,15 and 0,26 ml/min with a mean of $0{,}17 \pm 0{,}05$ ml/min or 0,05 ml/min/kg live mass. The lower flow rates at 40% SW may be an indication that salt gland stimulation was not maximal. No secretion occurred during the

first 30 minutes in the 40% SW group and the flow rate remained at about half the other rates for the rest of the collection time. It thus appears that the salt gland reacts to different osmotic loads by varying the flow rate. At higher dosage rates secretion increased with time and was maximal after 30 minutes.

The mean concentration of all the salt gland secretions was 1450±56 mOsm/l, but all the secretions during the first 30 minutes had lower osmotic, Na^+, K^+ and Cl^- concentrations than during the rest of the experiment. Decreased osmotic concentrations during the first 30 minutes were positively correlated with low flow rates ($r = 0,67$; $p = 0,05$). The relative composition of the major electrolytes of the salt gland secretion, however, remained the same at all treatments and times after dosing (Table 1). During the first 120 minutes after dosing, the salt gland disposed of approximately 5% of the volume load at the 100; 75 and 50% SW treatments but only half this figure at 40% SW. The percentage of the osmotic load

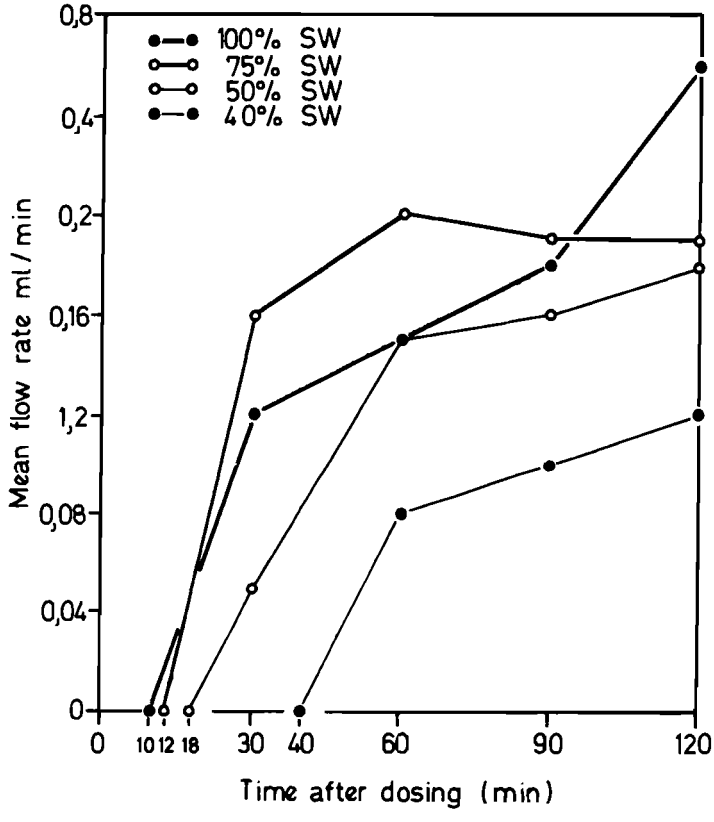

FIGURE 1
The effect of dosing various seawater concentrations on the mean secretion rate of the salt gland during the first 120 minutes after dosing. Seawater concentrations lower than 40% did not elicit secretion.

excreted via the salt gland during the same time increased from 6,3 ± 1,8% at 100% to 13,4% at 50% due to a decreased load and a relatively constant excretion. At 40% SW, however, the decreased flow rate induced a decrease in the percentage of the load excreted despite a lower osmotic load and a constant concentration of the salt gland fluid.

Cloacal excretion

Overall excretion via the cloaca decreased markedly as the solute load decreased despite the fact that a constant volume of fluid was dosed. The decrease in flow rate was most prominent during the first 30 minutes and to a lesser extent up to 60 minutes (Figure 2), but during the last 30 minutes of collection time this trend disappeared and treatments of 30% SW and lower had the highest flow rate (0,66 ± 0,11 ml/min vs 0,18 ± 0,13 ml/min, $p = 0,05$). From Figures 1 and 2 it would appear as if the cloacal flow pattern and salt gland activity showed some interdependence. At those concentrations where the salt gland was not stimulated (30;25;0% SW) the cloacal flow rate towards the end of the experimental period was significantly higher than the rate for the other treatments where the salt gland was active. It was also noted at the beginning of the experiment that the inhibition of cloacal excretion was considerably more obvious at those concentrations that were too dilute to elicit a salt gland response (Figure 2).

The ionic composition of cloacal fluid was affected by treatment (Table 2). Na^+ and Cl^-, but not K^+, decreased in relation to the osmotic concentration down to 30% SW, although there was a slight tendency for Na^+ to decrease at a greater rate, taking the percentage of the available osmotic space occupied by Na^+ as a guide. At 25% SW and lower, however, Na^+ decreased considerably more than Cl^- indicating a possible increase in selective absorption of Na^+. The mean K^+ concentration was 10,8 ± 1,3 meq/l at 40% SW and above but it decreased at lower concentrations. Na^+ decreased more than K^+ at the lower osmotic loads as the Na: K ratio decreased as well. This tendency for Na^+ to decrease more than the other ions was emphasized as the experiment progressed. Na^+ occupied 35,4 ± 6,1% of available osmotic space during the first 30 minutes for all treatments and this decreased to 26,2 ± 11,5% at 120 minutes. Further analysis of the data showed that 0% SW induced the most

TABLE 1

Composition of salt gland secretion as affected by time after dosing (mean ± SD)

Time after dosing (min.)	Osmotic concentration (mOsm)	ion concentration (meq/l)			Na: K	Na^+ as % of available osmotic space
		Na^+	K^+	Cl^-		
0 - 30	1128 ± 72	607 ± 49	21,9 ± 2,9	556 ± 53	27,7	53,8
31 - 120	1543 ± 54	760 ± 49	29,0 ± 2,5	666 ± 94	26,2	49,3

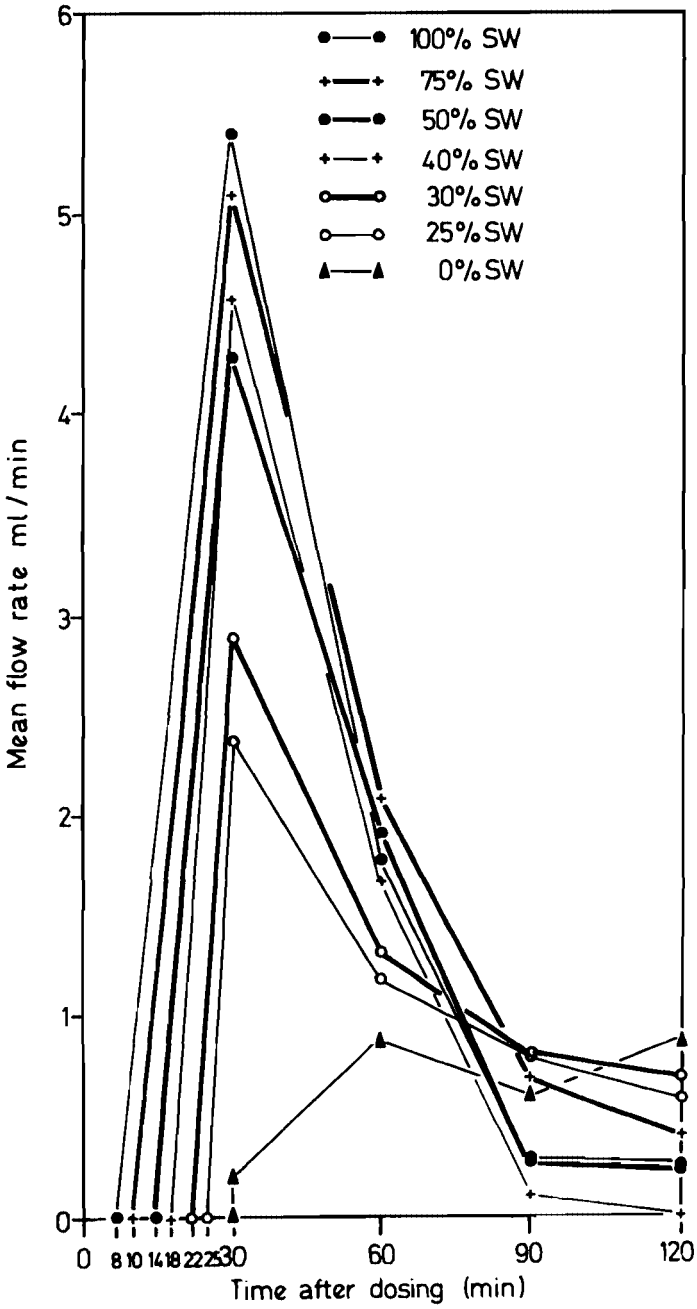

FIGURE 2

The effect of dosing various seawater concentrations on the mean excretion rate of the cloaca during the first 120 minutes after dosing.

dramatic retention of Na^+ as the contribution of Na^+ to total available osmotic space was decreased from 27% at 30 minutes to only 6,3% at 120 minutes. This decrease with time was less marked during the other treatments where the mean changed from 36,8 ± 5,3 to 30,2 ± 6,7% over 120 minutes. Cl^- occupied 40 - 45% of available osmotic space with no time or treatment effects.

The degree of change in composition of the fluid as it passed through the digestive tract is emphasized if the previous figures for the percentage of available osmotic space occupied by Na^+ are compared with the same parameter for seawater as the dosing fluid (43,7%). For Cl^-, the corresponding figure for seawater was 50,6% which indicates a smaller effect of the digestive tract on Cl^- when compared to Na^+.

The percentage of the solute load excreted via the cloaca increased with decreasing solute load from 28± 10,5% at 100% SW to 46,5± 3,8% at 30% SW because the osmotic concentration of cloacal fluid decreased less at lower dosing concentrations than the dosing fluid. At 25% it decreased again to 35,7± 6,3%.

Plasma samples could only be obtained from a few birds as a fairly low ambient temperature during sampling time had a constricting effect on the blood flow in the surface veins of the flipper where samples were taken. The mean ± S D of the data obtained are presented in Table 3. Dosing with seawater at concentrations considerably exceeding plasma concentrations, eg. 40% SW and above, increased the concentration of all plasma components although a large variation and low numbers per treatment prevented any of these differences from being statistically significant. Seawater concentrations approximating plasma concentrations (25 and 30% SW) did not affect plasma concentration whereas 0% SW had a depressing effect on all the measured parameters.

DISCUSSION

Salt gland excretion

In working with restrained wild birds the possibility of eliciting unnatural stress reactions from the salt gland should be kept in mind (McFarland 1965). In the present experiment, however, it was considered essential to use birds with solute handling abilities unaffected by hypo- or hypertrophic adaptation of the salt glands as may occur in captivity (Peaker & Linzell 1975), and as handling did not produce spontaneous secretion as recorded in some other birds (Fringe & Fringe 1959; Smith 1972; Hughes 1972), it is assumed that the results are valid for comparison of treatments.

From the present results it is concluded that solute loads just below 40% SW were the minimum required to stimulate the salt gland. From Figure 1 it would also appear that even 50% SW was not a maximum stimulus because not only was the onset of secretion retarded but flow rate only reached the level of the higher dosage rates after 60 minutes.

In the only other comparable experiment (i.e. oral loading with fluids of varying osmotic load but constant volume) Hughes (1972) loaded glaucous-winged gulls (*Larus glaucescens*)

TABLE 2

Composition of cloacal fluid as affected by treatment (mean ± SD)
*only one sample available, †sample not sufficient for this analysis

Treatment (% seawater)	Concentration (mOsm/l)	ion concentration (meq/l)			% of available osmotic space		Na: K ratio
		Na^+	K^+	Cl^-	Na^+	Cl^-	
100	435 ± 20	159 ± 10	10,2 ± 2,0	186 ± 4	36,6 ± 3,4	42,9 ± 3,8	17,2
75	416 ± 40	146 ± 17	10,3 ± 3,7	164 ± 12	36,8 ± 12,4	41,7 ± 2,7	20,1
50	329 ± 25	118 ± 5	9,9 ± 2,2	157 ± 8	36,4 ± 4,8	45,1 ± 2,4	14,3
40	361 ± 8	111 ± 16	12,8 ± 7,2	171 ± 5	35,5 ± 12,7	47,4 ± 3,5	16,1
30	329 ± 56	118 ± 20	7,5 ± 1,8	143 ± 19	34,6 ± 3,5	44,3 ± 3,6	16,2
25	285 ± 25	91 ± 12	7,4 ± 1,1	115 ± 12	31,8 ± 6,0	40,4 ± 3,6	12,6
0	160 ± 60	31 ± 17	3,3 *	†	15,8 ± 10		7,6

TABLE 3

Plasma composition of samples before and after treatment.

Treatment	Number of analyses	Concentration			
		Osmotic (mOsm/l)	Na^+ (meq/l)	K^+ (meq/l)	Cl^- (meq/l)
Before dosing	16	319 ± 6,8	146 ± 5,4	3,2 ± 0,8	115 ± 3,5
After dosing 40 - 100% SW	18	327 ± 15,9	157 ± 6,6	5,9 ± 3,0	119 ± 7,6
% change		+ 2,5	+ 7,5	+ 84,4	+ 2,6
After dosing 25 - 30% SW	3	320 ± 1,0	147 ± 3,5	4,0 ± 0,8	118 ± 4,9
% change		+ 0,3	+ 0,7	+ 25	+ 2,6
After dosing 0% SW	2	288 ± 5,6	133 ± 1,4	3,2 ± 1,5	109 ± 1,0
% change		- 9,7	- 8,9	0	- 5,2

with 23,5%; 32,5% and 65% seawater at 5% of body mass. (These concentrations have been recalculated by me in terms of the seawater analysis of the present experiment from the data given, 100; 150; and 300 meq/l NaCl, to make them more comparable with those of the present experiment). Whereas she reported spontaneous secretion after 45 minutes and even secretion after hyposmotic loads (after 77 minutes), no spontaneous secretion occurred in the present experiment and isosmotic (30% SW) and hyposmotic loads (25% and 0% SW) did not elicit any salt gland response even when administered at double the rate (10% of body mass). The penguin would therefore seem to use its salt gland only to dispose of hyperosmotic loads and it is not stimulated by the same factors operating on the glaucous-winged gull. The present results are, however, in full agreement with the osmoreceptor principle initially suggested by Schmidt-Nielsen (1960), and spontaneous secretion may be a handling artefact (Peaker & Linzell 1975) presumably only present in certain species. A final explanation is not possible at this stage because invariably different workers use different species with different stimulating regimes, and comparative studies are obviously called for.

Peaker *et al* (1973) tried to find the minimum mass of NaCl per kg body mass injected intravenously into various birds necessary to elicit a salt gland response. Their figures varied from 1,9 - 2,1 g/kg for the heavy goose (5,5 kg) through 8 - 29 g/kg for the duck (2 kg) to 6,1 - 10,6 g/kg for a gull of 1 kg. The comparable figures for the jackass penguin (3,7 kg) were close to !8,7 g NaCl per kg live weight when loaded orally with 40% SW, and these are within the reported range despite the different methods of loading. The percentage increase in blood plasma Na^+ concentration initiating salt gland secretion in birds of the size of penguins should be approximately 6% (Peaker & Linzell 1975) and the value of 7,5% in Table 3 is therefore within the range. Although the penguin seems to differ from the glaucous-winged gull in terms of the minimum stimulus required for the salt gland to function, it follows the same pattern generally found in birds with salt glands.

Maximum secretion rates (Figure 1) may take as long as 60 minutes to develop. Lower initial flow rates were positively related to the lower osmotic loads (40% and 50% SW), but this relationship disappeared when the stimulus was maximal (75% and 100% SW). It is therefore not surprising that experiments designed to stimulate the salt gland maximally by intravenous injection or feeding of very high salt concentrations failed to report this lag in reaching maximum activity. These lower initial flow rates were accompanied by lower concentrations (Table 1) although the relative composition of the major electrolytes remained constant. This positive correlation ($r = 0,67$; $p = 0,05$) between flow rate and concentration is in agreement with a similar trend reported by Hanwell *et al.* (1971) for data pooled from various geese.

It thus appears that the salt gland reacts to different marginal osmotic loads by varying the flow rate and total concentration of the secretion to match the degree of osmotic stress. At higher osmotic loads the secretion rate and osmotic concentration reached maximum values within 30 minutes and remained high thereafter, thus working at a maximum rate to eliminate the high osmotic loads. The relative composition of the various ions, however, remained unchanged.

Cloacal excretion

This component is derived from secretions of the kidney and digestive tract modified by the cloaca. From the data of Erasmus (in press) the relative contribution of these two organ systems can be expected to vary with osmotic load, and interpretation of the data on flow rate and composition of the final product should take this into consideration.

The decrease in cloacal excretion with a decrease in osmotic load, despite a constant volume dosed, argues against interpreting high cloacal flow rates as due to mechanical fill. It supports my conclusion (Erasmus in press) that the digestive tract and cloaca play an important regulatory role in disposing of excess osmotic loads, and it is also in agreement with Holmes *et al.* (1968) who concluded that the cloacal epithelium may be "sensitive to hypertonic urine". The cloacal control (Peaker & Linzell 1975) can therefore be interpreted as a graded and controlled reaction, with the magnitude depending on the degree of osmotic stress.

The cloacal system appears to be stimulated to excrete by the same stimuli as the salt gland. Cloacal flow rates at those dosing rates that did not stimulate the salt gland to secrete, were significantly lower during the first part of the experiment than at those dosing levels where the salt gland did function. This suggests a possible mechanism whereby the activity of the salt gland and digestive tract is co-ordinated. Towards the end of the experiment, however, this relationship in flow rates between the two organs was reversed, possibly due to an increased urine, and therefore solute, inflow into the cloaca.

The composition of the cloacal fluid relative to the dosing fluid suggests that the dosing fluid tends to equilibrate with plasma, and the change in Na^+ concentration supports the general concept of selective Na^+ absorption by the cloaca and digestive tract (Douglas 1970; Erasmus in press). This tendency increased as the osmotic load decreased, especially where the Na^+ concentration of the dosing fluid was less than that of the plasma, and may be due to low solute loads as the digestive tract would therefore have more time to absorb Na^+.

No other ions (eg. NH_4^+) were monitored, but Na^+ and Cl^- together occupied as much as 80,3 $\pm$ 1,9% of available osmotic space at treatments of 30% SW and higher. (With K^+ this figure becomes 83,0 $\pm$ 2,4%). At lower dosing rates with lower flow rates, however, Na^+ took up a smaller proportion of the available osmotic space. The nature of the unknown cation taking over from Na^+ is not known, but from the results of Douglas (1970) and Erasmus (in press) it is most probably NH_4^+. If this is the case, then it follows that NH_4^+ should only feature as an important ion in those cases where the need for Na^+ absorption is high. This should be the case during active salt-gland secretion.

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