
REVIEW

OSMOREGULATION IN MARINE MAMMALS

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Summary

Osmoregulation in marine mammals has been investigated for over a century; however, a review of recent advances in our understanding of water and electrolyte balance and of renal function in marine mammals is warranted. The following topics are discussed: (i) kidney structure and urine concentrating ability, (ii) sources of water, (iii) the effects of feeding, fasting and diving, (iv) the renal responses to infusions of varying salinity and (v) hormonal regulation. The kidneys of pinnipeds and cetaceans are reniculate in structure, unlike those of terrestrial mammals (except bears), but this difference does not confer any greater concentrating ability. Pinnipeds, cetaceans, manatees and sea otters can concentrate their urine above the concentration of sea water, but only pinnipeds and otters have been shown to produce urine concentrations of Na⁺ and Cl⁻ that are similar to those in sea water. This could afford them the capacity to drink sea water and not lose fresh water. However, with few

exceptions, drinking is not a common behavior in pinnipeds and cetaceans. Water balance is maintained in these animals *via* metabolic and dietary water, while incidental ingestion and dietary salt may help maintain electrolyte homeostasis. Unlike most other aquatic mammals, sea otters commonly drink sea water and manatees frequently drink fresh water. Among the various taxonomic groups of marine mammals, the sensitivity of the renin–angiotensin–aldosterone system appears to be influenced by the availability of Na⁺. The antidiuretic role of vasopressin remains inconclusive in marine mammals, while the natriuretic function of atrial natriuretic peptide has yet to be examined. Ideas on the direction of future studies are presented.

Key words: kidney, urine, water, feeding, fasting, diving, salinity, drinking, aldosterone, vasopressin, angiotensin.

Introduction

The regulation of water and electrolyte levels in marine mammals has been a topic of research spanning the last century (for references, see Fetcher, 1939). However, this is the most recent review dedicated solely to a discussion of osmoregulation in marine mammals. Since then, a number of significant contributions to the field have been made that have greatly enhanced our current understanding of water and electrolyte metabolism and of renal function in marine mammals. The present review was prepared to help summarize the last 100 years of study of osmoregulation in marine mammals.

Marine mammals are well adapted to their hyperosmotic environment. To osmoregulate properly in a marine habitat, physiological mechanisms intended to conserve fresh water and thus avoid dehydration are required. However, as challenging as enduring life in a high-salt environment may be, aquatic mammals also diverged and inhabited freshwater niches. Although adapting to freshwater habitats may appear

beneficial because conserving fresh water no longer poses a problem, those aquatic mammals that adopted such habitats are confronted with a different osmotic challenge. Living in a salt-‘free’ environment requires the appropriate physiological mechanisms to conserve electrolytes. However, some marine mammals have adapted to yet a third, and potentially greater, osmotic challenge, prolonged fasting in an arid terrestrial environment (i.e. Ortiz et al., 1978). The ability to maintain water balance and electrolyte homeostasis during extended periods of complete abstinence from food and water requires even more robust mechanisms designed to conserve both salts and water while maintaining internal homeostasis. Occupying either extreme in environmental salinity, or adapting to both, as well as adapting to prolonged periods of fasting provide an indication of the dynamic scope in osmoregulatory capacity of marine mammals, which has made them an intriguing model for the study of renal function for over a century.

Kidney structure

The difference in kidney structure between marine and terrestrial mammals suggests that the 'specialized' kidneys of marine mammals allow them to occupy habitats with a broad range of salinity, since the kidneys are the principal organs of water and electrolyte regulation. Members of the order Cetacea (dolphins and whales) (Hedges et al., 1979; Pfeiffer, 1997) and Pinnipedia (seals, sea lions and walruses) (Bester, 1975; Vardy and Bryden, 1981) possess reniculate kidneys similar to those of bears and otters. These kidneys can be made up of hundreds of individual lobes, or reniculi, each of which contains discrete cortical tissue and a single medullary pyramid inserted in a single calyx (Bester, 1975; Vardy and Bryden, 1981). In manatees (order Sirenia), the kidney is considered superficially lobulate since it lacks true reniculi and the cortex is continuous (Maluf, 1989).

A histological dissection of the cetacean kidney reveals structures probably adapted to accommodate diving behavior (Pfeiffer, 1997). Among the structures identified in cetacean kidneys that are lacking in those terrestrial mammalian kidneys studied are (i) specialized glycogen stores in the proximal convoluted tubule epithelial cells, (ii) highly concentrated bundles of medullary blood vessels (vasa recta bundles) and (iii) the presence of a sporta perimedullaris musculosa, a layer of collagen, elastic fibers and smooth muscle separating the cortex from the medulla (Pfeiffer, 1997).

The anatomical structure of the kidney in sirenians differs from that of pinnipeds and cetaceans (Bester, 1975; Hedges et al., 1979; Maluf, 1989). Sirenians range from the strictly marine dugong (*Dugong dugon*) to the strictly freshwater Amazonian manatee (*Trichechus inunguis*) to the West Indian (*T. manatus*) and West African (*T. senegalensis*) manatees, which inhabit both fresh- and salt-water habitats. The kidney of dugongs has been described as externally elongated, with features similar to those found in the kidneys of the camel and horse (Batrawi, 1957), while the kidney of West Indian manatees has several large lobes with a continuous cortex (Maluf, 1989). The striking differences in renal anatomy among the various species of sirenians suggest that these variations in kidney morphology may be related to the variations in habitat of each species.

Kidneys in marine mammals possess the anatomical prerequisites (i.e. increased medullary thickness) necessary to produce a highly concentrated urine, which is especially important for mammals in a hyperosmotic environment. However, as stated by Bester (Bester, 1975), 'the reniculate kidney does not attain the concentrating ability expected from a kidney with such excellent anatomical credentials'. A close correlation between relative medullary thickness and maximum recorded urine osmolality exists, with pinnipeds exhibiting ratios between 1.1 and 1.7 (Vardy and Bryden, 1981). However, these low values suggest that the kidneys of marine mammals are extremely poor concentrators of urine (Vardy and Bryden, 1981). Using a urine-to-plasma ratio in osmolality as an index of concentrating ability, values for marine mammals are only slightly higher than those for

Table 1. Comparison of urine osmolality and urine-to-plasma ratios as indices of concentrating ability among various terrestrial and marine mammals

	Urine osmolality (mosmol l ⁻¹)	Urine: plasma ratio	Source
Terrestrial			
Human	1400	4.6	Vander, 1995
Dog	1800	6	DiBartola et al., 1980
Camel	2800	7	Ben Goumi et al., 1993
Domestic cat	3100	10	Schmidt-Nielsen, 1990
Kangaroo rat	5500	14	Schmidt-Nielsen, 1990
Hopping mouse	9400	25	Schmidt-Nielsen, 1990
Marine/aquatic			
West Indian manatee	1158	3.7	Irvine et al., 1980; Ortiz et al., 1998
Sei whale	1353	3.8	Fetcher, 1939
American river otter	1482	4.8	Hoover and Tyler, 1986
Rough-toothed dolphin	1700	5.0	Malvin and Rayner, 1968
Bottlenose dolphin	1815	5.3	Malvin and Rayner, 1968
Weddell seal	1760	5.6	Kooyman and Drabek, 1968
Northern elephant seal	1850	5.9	Ortiz et al., 1996
Grey seal	2161	6.0	Skog and Folkow, 1994
Harbor seal	2050	6.2	Page et al., 1954; Tarasoff and Toews, 1972
Sea otter	2130	6.7	Costa, 1982
Ringed seal	2420	6.8	Portier, 1910
Baikal seal	2374	6.9	Hong et al., 1982
Cape fur seal	2364	7.0	Bester, 1975

Data for marine mammals were used only if plasma osmolality was also available in the same or another reference.

The West Indian manatee, American river otter and Baikal seal are freshwater species.

humans, but much lower than those for desert rodents (Table 1). Therefore, the variation in kidney morphology observed in marine mammals does not appear to afford them any greater benefit than terrestrial mammals, suggesting that the adaptation of mammals to a hyperosmotic environment was accomplished *via* more conventional mechanisms such as hormonal regulation of urine concentration and/or the rate of urine formation. The reniculate kidneys of cetaceans and pinnipeds probably evolved in response to their large body size and diving abilities and not to the osmotic challenge posed by a marine environment (Bester, 1975; Vardy and Bryden, 1981).

Urine concentration

Although the consumption of sea water is not a common behavior in marine mammals in general (with a few

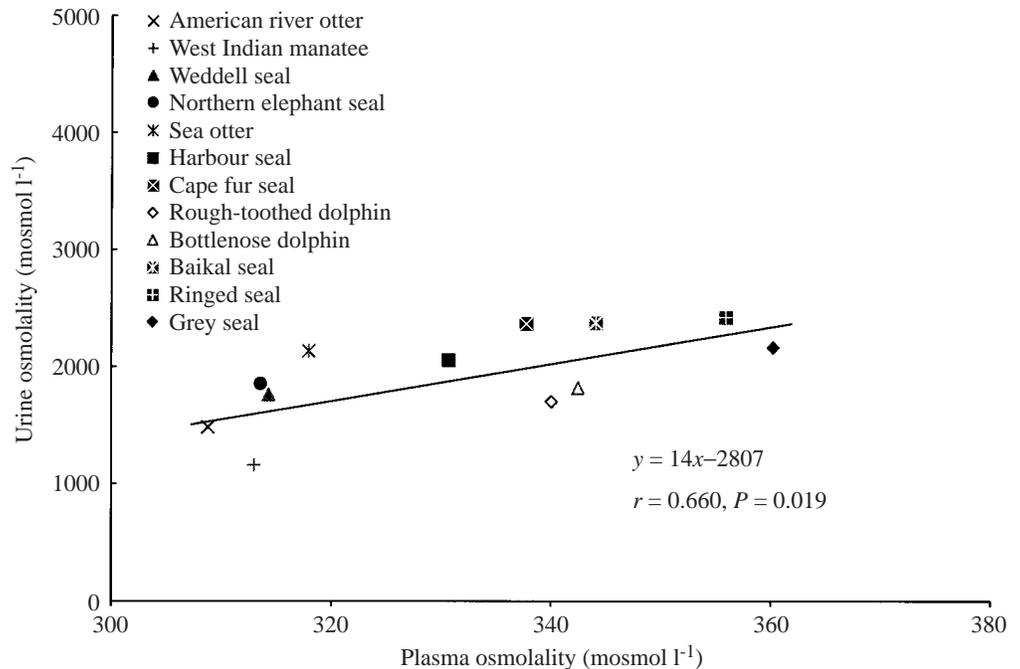


Fig. 1. Correlation between plasma and urine osmolality for various marine mammals. The freshwater species are the West Indian manatee (*Trichechus manatus*), American river otter (*Lutra canadensis*) and Baikal seal (*Phoca sibirica*). See Table 1 for references. The regression was considered significant at $P < 0.05$.

exceptions), these animals can produce urine with an osmolality greater than that of sea water (Bester, 1975; Costa, 1982; Maluf, 1989). The highest urine osmolality measured in any marine mammal was $2658 \text{ mosmol l}^{-1}$ (Ridgway, 1972). Even mammals found in freshwater habitats can concentrate their urine in response to a hyperosmotic stimulus (Irvine et al., 1980; Hong et al., 1982), suggesting that marine mammals that are found in freshwater rather than marine environments maintained their urine-concentrating abilities (Table 1). The increase in urine osmolality is associated with increased plasma osmolality, as indicated by the positive correlation between plasma and urine osmolality in marine mammals (Fig. 1).

An examination of the cortical:medullary thickness of the kidney in West Indian manatees suggests that these animals possess the ability to concentrate their urine to an osmolality greater than that of sea water (Hill and Reynolds, 1989; Maluf, 1989) and, in fact, urine osmolalities of approximately $1200 \text{ mosmol l}^{-1}$ have been determined from samples taken from wild animals in salt water (Irvine et al., 1980). When manatees that had been held in fresh water were exposed to salt water for 4 days, the sole urine sample collected was only $217 \text{ mosmol l}^{-1}$, 29% lower than the animal's plasma osmolality (Ortiz et al., 1998). Although the animal was in salt water, it continued to feed on its normal diet of lettuce (which has a high water content), so a source of fresh water was maintained and there was no requirement to concentrate its urine (Ortiz et al., 1998).

Originally, it was believed that marine mammals drank sea water because their urine osmolality was greater than that of sea water, and it was assumed that their urine was essentially composed of Na^+ and Cl^- (Portier, 1910). However, for marine mammals to obtain a net positive gain in solute-free water after

consuming sea water, they would have to excrete Na^+ and Cl^- in concentrations greater than that in the water they drink (Albrecht, 1950). With few exceptions, marine mammals do not regularly concentrate Na^+ and Cl^- to levels above that of sea water (Table 2), further suggesting that they do not rely on the consumption of sea water to maintain their fluid balance.

Seals and sea lions

The majority of the studies on water and electrolyte balance in marine mammals have used pinnipeds, so most of the generalizations made about osmoregulation in marine mammals are derived from these data. Investigations of renal function during feeding and fasting, as well as in response to infusions of varying salinity and to simulated diving (forced apnea), have been conducted. More information on hormonal regulation of kidney function exists for pinnipeds than for any of the other marine mammal groups, but the data are still limited.

Metabolic water and drinking

The fact that seals can maintain water balance solely from pre-formed water in their diet and the subsequent metabolically derived water was recorded in the 1930s (Irving et al., 1935; Smith, 1936; Fetcher, 1939). This has subsequently been verified in later studies (i.e. Pilson, 1970; Depocas et al., 1971; Ortiz et al., 1978).

Although voluntary drinking of salt water (mariposia) has not been measured empirically in phocids (true seals), ingestion of sea water accounted for 9.2% and 7.25%, respectively, of the total water flux rate of fed and unfed harbor seals (*Phoca vitulina*) (Depocas et al., 1971) (Table 3). The authors concluded that this amount was not a quantitatively

Table 2. *Maximum urinary electrolyte and urea concentrations compared with sea water*

Order Common name	Na ⁺ (mmol l ⁻¹)	K ⁺ (mmol l ⁻¹)	Cl ⁻ (mmol l ⁻¹)	Urea (mmol l ⁻¹)	Source
Sea water	470	10	548	NR	Barnes, 1954
Pinnipedia					
Baikal seal	244	137	202	1817	Hong et al., 1982
California sea lion	442	118	608	65	Pilson, 1970; Ridgway, 1972
Cape fur seal	368	216	567	1640	Bester, 1975
Harbor seal	523	370	508	NR	Bradley et al., 1954; Tarasoff and Toews, 1972
Northern elephant seal	80	230	NR	490	Adams and Costa, 1993; Ortiz et al., 1996
Northern fur seal	160	129	140	833	Keyes et al., 1971
Ringed seal	297	157	267	1351	Hong et al., 1982
Weddell seal	330	125	NR	1248	Kooyman and Drabek, 1968
Cetacea					
Bottlenose dolphin	460	179	632	1345	Ridgway, 1972
Beluga whale	NR	NR	39	570	Fetcher, 1939
Blue whale	NR	NR	450	NR	Fetcher, 1939
Finback whale	330	72	850	430	Fetcher, 1939
Pilot whale	263	NR	NR	NR	Telfer et al., 1970
Sei whale	330	82	370	650	Fetcher, 1939
Sirenia					
West Indian manatee	31	60	406	NR	Ortiz et al., 1998
Fissipedia					
American river otter	31	19	16	NR	Hoover and Tyler, 1986
Sea otter	505	117	555	953	Costa, 1982

NR, not reported.

Table 3. *Comparison of mean water flux rates among various marine mammals*

Order Common name	Age class	Condition	N	Water flux (ml kg ⁻¹ day ⁻¹)	Source
Pinnipedia					
Grey seal	Pup	Fasting	5	6±1	Nordøy et al., 1992
Harbor seal	Yearling	Fed	5	51±15	Depocas et al., 1971
		Fasted	2	18±2	Depocas et al., 1971
Northern elephant seal	Pup	Fasting	5	6±1	Ortiz et al., 1978
Ringed seal	Pup	Nursing	3	63±22	Lydersen et al., 1992
Weddell seal	Pup	Nursing	14	36±6	Tedman and Green, 1987
Cetacea					
Common dolphin	Subadult	Fasted	2	77±1	Hui, 1981
Harbor porpoise	Subadult	Fed	1	425±35	Andersen and Nielsen, 1983
Pilot whale	Adult	Fasted	3	4.5	Telfer et al., 1970
Sirenia					
West Indian manatee	Adult	Fed in FW	4	145±12	Ortiz et al., 1999
		Fed in SW	2	45±3	Ortiz et al., 1999
		Fasted in SW	5	17±2	Ortiz et al., 1999
Fissipedia					
Sea otter	Adult	Fed	5	269±50	Costa, 1982

Values are means ± s.d.

FW, fresh water; SW, sea water.

important component of water intake and was probably the consequence of incidental ingestion during feeding under water (Depocas et al., 1971). Mariposia has also been observed

in captive harbor seals, but the amount could not be quantified (Ridgway, 1972). Observations of 'drinking' behavior have been reported, primarily in males, of at least six of the 14

otariid (eared pinnipeds) species (Gentry, 1981). The conclusions drawn by Gentry (Gentry, 1981) do not refute the generalization that mariposia is not usually required to maintain water balance in marine mammals, but suggest that mariposia may be common in fasting animals inhabiting warm environments as a means of assisting with nitrogen excretion and thermal regulation (Gentry, 1981). Seawater drinking ($18.3 \pm 3.4 \text{ ml kg}^{-1} \text{ day}^{-1}$, mean \pm S.E.M., $N=3$) occurs in Galápagos fur seals (*Arctocephalus galapagoensis*), which inhabit a tropical environment, but not in Antarctic (*A. gazella*) or northern (*Callorhinus ursinus*) fur seals (Costa and Gentry, 1986; Costa and Trillmich, 1988), which are found in colder regions. Drinking in otariids appears to be dictated by environmental temperature as a means to counter thermal stress.

Ingestion of fresh water has been reported in harp seals (*Phoca groenlandica*) in the form of ice consumption (Renouf et al., 1990). Irving et al. (Irving et al., 1935) observed harbor seals drinking fresh water 'greedily' as soon as it was made available following transport in a 'warm express car'. In another instance, drinking of fresh water was induced in a harbor seal after it had been infused with 1 l of sea water (Albrecht, 1950). However, the significance of fresh water to the maintenance of fluid balance has yet to be elucidated. Collectively, metabolic and dietary water should be sufficient to maintain water balance, and drinking either salt or fresh water does not appear to be a common behavior in phocids.

Drinking is not essential for maintaining water balance, but the incidental ingestion associated with feeding may be important for maintaining electrolyte homeostasis and may explain the observations of otariids drinking. Hyponatremia may be induced in captive pinnipeds maintained in fresh water and fed a salt-restricted diet and, in some instances, they may succumb to it (Geraci, 1972). Therefore, dietary salt supplements for captive pinnipeds held in fresh water or on low-salt diets may be important to help avoid hyponatremia.

Fasting and feeding

Fasting is a natural component of the life history of pinnipeds, but the duration of the fast varies among species (for a review, see Riedman, 1990). A comparison of mass-specific water flux and the biological half-time ($t_{1/2}$) of water between naturally fasting northern elephant seal (*Mirounga angustirostris*) pups and force-starved harbor seals demonstrated a water flux rate (Table 3) that was three times lower and a $t_{1/2}$ that was 2.5 times higher in the elephant seal (Depocas et al., 1971; Ortiz et al., 1978). On the basis of these differences, elephant seals appear to be better adapted to prolonged periods of food-deprivation than harbor seals, as suggested previously (Ortiz et al., 1978). Naturally fasting grey seal pups exhibited similar mass-specific efflux rates to those of fasting elephant seal pups and a slightly lower $t_{1/2}$ (Nordøy et al., 1992). The low turnover rates of water allow elephant and grey seal pups to maintain their water balance during their prolonged (1.5–3 months) periods of fasting (Table 3).

The maintenance of electrolyte homeostasis during the

postweaning fast in elephant seal pups was attributed to an increase in renal resorption of Na^+ and K^+ at the expense of H^+ , which may alleviate metabolic acidosis (Ortiz et al., 2000). Postweaned harp seal pups, which naturally fast for approximately 6 weeks, maintained ionic homeostasis and hydration state following 10 weeks of forced fasting (Worthy and Lavigne, 1982). However, fasting grey seal pups did not maintain osmotic and ionic homeostasis to the same degree as fasting elephant and harp seal pups (Ortiz et al., 1978; Nordøy et al., 1992; Ortiz et al., 2000). The increases of 7.5 % in plasma $[\text{Na}^+]$ and of 35 % in plasma osmolality exhibited by grey seal pups at the end of their 52 day fast suggest some degree of dehydration, although percentage total body water actually increased by 7 %, indicating that water balance had been maintained (Nordøy et al., 1992). Clinically, increases in hematocrit (Hct, %) and plasma osmolality are used in human medicine as initial indicators of dehydration (Shirreffs, 2000). However, Hct measurements in pinnipeds are quite variable, making their interpretation difficult (Castellini et al., 1996). For example, Hct increased by 35 % over the postweaning fast in northern elephant seal pups (Ortiz et al., 2000), but did not change in fasting harp seal pups (Worthy and Lavigne, 1982). However, other indicators of dehydration such as increased plasma osmolality, electrolyte levels and total protein levels (Ortiz et al., 2000) and decreased percentage total body water (Ortiz et al., 1978; Houser et al., 2001) and circulating water content (Castellini et al., 1990) were not observed in elephant seal pups. The increase in Hct has been attributed to an increase in red blood cell volume associated with the development of the diving ability of this species (Kohin, 1998). Therefore, changes in Hct are independent of hydration state in pinnipeds.

The oxidation of fat produces proportionately more water than the oxidation of other substrates, allowing fasting seals to rely primarily on water derived metabolically from the catabolism of fat stores as their sole source of water (Ortiz et al., 1978; Castellini et al., 1987). Therefore, water conservation mechanisms must be extremely effective in fasting seals to maintain body water content. During the 2–3 month fasting period of postweaned elephant seal pups, water balance is maintained by a reduction in the nitrogen (primarily urea) load on the kidneys by means of a decrease in protein catabolism (Adams and Costa, 1993) and by an increase in urine osmolality (Ortiz et al., 1996), which collectively result in a decrease in urinary water loss (Adams and Costa, 1993; Ortiz et al., 1996). An increase in urine osmolality was also measured in starved harbor seals, further suggesting an increase in water conservation *via* urinary concentrating mechanisms (Skog and Folkow, 1994). A reduced glomerular filtration rate (GFR), associated with an increase in tubular resorption of water, further reduces water loss in fasting elephant seal pups (Pernia et al., 1989; Adams and Costa, 1993). However, the situation for fasting, lactating elephant seals appears to be quite different from that in their pups. Between mid and late lactation, an increase in absolute and mass-specific GFR was observed in nursing mothers, associated with an increase in protein catabolism (Crocker et

al., 1998). The increase in GFR was probably the result of an increase in glomerular oncotic pressure associated with an increase in nitrogen-loading on the kidneys. Although an increased GFR usually results in an increase in excreted water (or decreased resorption of water), fractional resorption of urea was calculated to increase, thus reducing urinary water loss despite the increase in GFR (Crocker et al., 1998).

Body water may be further conserved (during the fast) by a significant reduction in respiratory water loss using nasal countercurrent heat exchange (Huntley et al., 1984; Folkow and Blix, 1987). Although nasal heat exchange was not determined to be an effector mechanism for regulation of water balance (Skog and Folkow, 1994), in the absence of nasal heat exchange, 10–33% of total water flux would be lost (Folkow and Blix, 1987), suggesting the potential importance of nasal heat exchange in reducing respiratory water loss in seals.

Feeding increased GFR and renal plasma flow (RPF) in the harbor seal, while filtration fraction (the fraction of the plasma filtered through the glomeruli; GFR/RPF) remained constant (Hiatt and Hiatt, 1942; Schmidt-Nielsen et al., 1959). The effective stimulus resulting in an increase in RPF was not an increase in available water, but was probably an increase in nitrogen-loading (urea) on the kidney associated with a high-protein diet (Hiatt and Hiatt, 1942). Mass-specific GFR in harbor seals varied with RPF between fasting ($0.2 \text{ ml min}^{-1} \text{ g}^{-1}$ kidney mass) and feeding ($1.0 \text{ ml min}^{-1} \text{ g}^{-1}$ kidney mass) (Hiatt and Hiatt, 1942), but mass-specific GFR did not change between periods of nursing and fasting in elephant seal pups (Houser et al., 2001). If the mechanisms linking GFR and RPF in elephant seals are the same as those in harbor seals, then the absence of a change in GFR between nursing and fasting may be the result of the low protein content of their milk (on average less than 8%) (Riedman and Ortiz, 1979; Ortiz et al., 1984). Therefore, changes in GFR, and probably in RPF, in pinnipeds appear to be influenced by dietary protein content.

Feeding has also been shown to increase urinary Na^+ , K^+ and urea concentrations (Schmidt-Nielsen et al., 1959). Increased urea excretion was attributed to an elevation in plasma urea concentration, GFR and urine flow rate (Schmidt-Nielsen et al., 1959). However, the increase in GFR observed after feeding had no apparent effect on the fraction of filtered urea that was excreted (Schmidt-Nielsen et al., 1959). This, together with the low urine:plasma ratio of urea, indicates that the harbor seal's ability to concentrate urea is poor (Ladd et al., 1951; Schmidt-Nielsen et al., 1959), suggesting that the kidney of the harbor seal has a low capacity to transport urea.

Smith (Smith, 1936) demonstrated in the harbor seal that simultaneous renal clearances of inulin and creatinine were essentially identical, indicating that the error associated with the creatinine clearance method induced by the secretion of creatinine by the kidney into the filtrate is insignificant and minimal in these seals. This was important because it provided researchers with two independent methods for measuring GFR, allowing for quantitative comparisons of GFR obtained using the two techniques.

Typically, fasting animals rely primarily on the metabolism

of fat and, in some instances, protein stores and associated pre-formed water (Worthy and Lavigne, 1987) to maintain their water balance, whereas feeding animals have the advantage of using not only the water produced from the metabolism of their food but also the pre-formed water in their diet. Because water is generally more available during feeding, the activity of the kidney is increased, allowing for increased excretion of dietary nitrogen and electrolytes. During fasting, the activity of the kidney is reduced and the resorption of endogenous electrolytes and body water is increased to help maintain fluid and electrolyte homeostasis. In the case of fasting grey seals, plasma osmolality increases just prior to the end of the fast, and this does not appear to have any deleterious effects on hydration state (Nordøy et al., 1992).

Solute and water loading and infusions

A number of infusion studies have been performed to challenge the dynamic scope of renal capacity in pinnipeds. Fresh water, hyperosmotic saline plus mannitol and hyperosmotic KCl have been administered to examine the effects of changes in filtration associated with these infusions on urinary excretion of water and electrolytes. Infusion of isotonic gelatine, which increases plasma volume without changing osmolality, has also been performed to determine the effects of changes in volume on filtration and excretion.

Water-loading experiments were achieved primarily *via* stomach tube (Albrecht, 1950; Ladd et al., 1951; Bradley et al., 1954; Tarasoff and Toews, 1972; Hong et al., 1982; Skog and Folkow, 1994), with one exception in which intravenous injection was employed (Murdaugh et al., 1961a). Differences in infusion rates and volumes may contribute to some of the discrepancies among the various studies (Table 4). It is also worth noting that a number of harbor seals have died as a consequence of water intoxication caused by excessive loading (Ladd et al., 1951).

Freshwater loads increase urine flow and decrease urine osmolality within 2 h (Albrecht, 1950; Bradley et al., 1954; Tarasoff and Toews, 1972; Hong et al., 1982; Skog and Folkow, 1994). These changes in urine output and osmolality are coincident with a decrease in plasma osmolality, but there is no change in plasma vasopressin levels (Skog and Folkow, 1994). Water-loaded harbor seals exhibited a decrease in excreted Na^+ and K^+ (Bradley et al., 1954) and no significant change in GFR (Bradley et al., 1954; Murdaugh et al., 1961a). However, Ladd et al. (Ladd et al., 1951) induced diuresis, which was attributed to a decrease in tubular, free water resorption and an increase in GFR. Increased diuresis in response to water loading in Baikal (*Phoca sibirica*) and ringed (*P. hispida*) seals resulted in an increased clearance of Na^+ , K^+ , Cl^- and urea, indicating a reduction in the percentage of the filtered load that is reabsorbed (Hong et al., 1982). Maximum urine flow during water diuresis has been shown to represent between 10 and 12% of GFR, indicating that only 88–90% of filtered water is reabsorbed following infusions of fresh water (Ladd et al., 1951; Hong et al., 1982). This decrease in fractional clearance of water suggests that water resorption

Table 4. A summary of infusion protocols used in pinnipeds and cetaceans

Order Species	Body mass (kg)	Route	Infusate	Dose (l)	Rate of infusion (ml min ⁻¹)	Source
Pinnipedia						
California sea lion	39	Oral	FW	1.5	NR	Ridgway, 1972
Harbor seal	16–31	Oral	FW	50–150*	NR	Ladd et al., 1951
Grey seal	44–52	Oral	FW	1.5	NR	Skog and Folkow, 1994
Harbor seal	30	iv	SW	2.6	11–33	Albrecht, 1950
Baikal seal	46	iv	0.5 mol l ⁻¹ NaCl	0.5	16.7	Hong et al., 1982
Ringed seal	72	iv	0.5 mol l ⁻¹ NaCl	0.5	16.7	Hong et al., 1982
Harbor seal	30	Oral	FW and SW	1	NR	Albrecht, 1950
Harbor seal	45	Oral	FW and SW	0.5–1	NR	Tarasoff and Toews, 1972
Baikal seal	46, 55	Oral	FW and 0.15 mol l ⁻¹ NaCl	1 0.5	NR NR	Hong et al., 1982
Ringed seal	68, 72	Oral	FW and 0.15 mol l ⁻¹ NaCl	1 0.5	NR NR	Hong et al., 1982
Harbor seal	14–18	Oral	FW, NaCl, KCl	0.3	NR	Bradley et al., 1954
Harbor seal	20–25	iv	1 % gelatine	0.4	8	Murdaugh et al., 1961a
Grey seal	44–52	iv	930 mmol l ⁻¹ mannitol	1	8.3	Skog and Folkow, 1994
Cetacea						
<i>Tursiops gilli</i>	160	Oral	FW	4	NR	Malvin and Rayner, 1968
<i>T. truncatus</i>	187	Oral	FW	4	NR	Ridgway, 1972
<i>T. truncatus</i>	120	Oral	0.5 mol l ⁻¹ NaCl	2	NR	Fetcher and Fetcher, 1942
<i>T. truncatus</i>	101, 177	Oral	SW	1, 3	NR	Ridgway, 1972

Oral, *via* a stomach tube; iv, intravenous; FW, fresh water; SW, salt water; NR, not reported.

*Values in ml kg⁻¹.

decreases as GFR increases (Ladd et al., 1951). In two instances, seals were reported to have produced hypo-osmotic urine following infusion of fresh water (Ladd et al., 1951; Hong et al., 1982). Overall, loading of fresh water results in an increase in urine volume and fractional clearance of water and electrolytes, attributed to an increase in GFR, and a decrease in plasma and urine osmolality.

Hyperosmotic solutions were introduced primarily *via* a stomach tube (Albrecht, 1950; Bradley et al., 1954; Ridgway, 1972; Hong et al., 1982) with a few exceptions (Albrecht, 1950; Hong et al., 1982). Following an infusion equivalent to 3.3 % of body mass in a harbor seal, copious vomiting and diarrhea occurred, resulting in a net loss of body water (Albrecht, 1950). Vomiting and diarrhea were also observed in California sea lions fed salt tablets in their diet (Ridgway, 1972). Continuous intravenous infusion of hyperosmotic saline resulted in the death after 77 and 171 min of two harbor seals (approximately 30 kg), suggesting that marine mammals do not have any greater resistance to seawater toxicity than terrestrial mammals (Albrecht, 1950). Although infusates were primarily NaCl solutions, KCl was used in three harbor seals, one of which died of K⁺ intoxication (Bradley et al., 1954).

Infusions of hyperosmotic solutions of varying concentrations are associated with an increase in urine flow and excreted Na⁺ and Cl⁻ (or urine osmolality in the case of mannitol) as soon as 40 min after infusion (Albrecht, 1950; Bradley et al., 1954; Tarasoff and Toews, 1972; Hong et al.,

1982; Skog and Folkow, 1994). Infusion of mannitol resulted in a decrease in plasma electrolyte and urea concentrations, although plasma osmolality was not significantly altered (Skog and Folkow, 1994). Skog and Folkow (Skog and Folkow, 1994) calculated that the excretion of osmotic solutes following the infusion of mannitol required 2.5 % of total body water. The increase in urine output was shown to be dose-dependent (Tarasoff and Toews, 1972). Calculations made from urine output and urine osmolality data (Tarasoff and Toews, 1972) indicate that osmotic excretion is also dose-dependent. The increase in urine flow was not associated with an increase in GFR, as with infusions of fresh water (Bradley et al., 1954). Urinary Cl⁻ concentration was also elevated (above that of sea water) following infusion of hyperosmotic saline (Albrecht, 1950).

Intravenous infusion of isotonic gelatine (Murdaugh et al., 1961a) was used to investigate the effects of plasma volume expansion on renal hemodynamics. Infusion of gelatine increased urine volume and osmotic excretion (Murdaugh et al., 1961a), resulting in solute diuresis associated with an increase in GFR with no change in plasma osmolality. These changes led Murdaugh et al. (Murdaugh et al., 1961a) to suggest that a volume receptor is present in harbor seals that is regulated differently from that in humans.

Apnea/simulated diving

Bradley and Bing (Bradley and Bing, 1942) published the

first study of the effects of apnea (forced diving) on renal hemodynamics using the harbor seal. They demonstrated that, during periods of apnea and forced diving, GFR and RPF are reduced while the filtration fraction (GFR/RPF) remains relatively constant. Later, it was shown that the apnea-induced decrease in GFR was also associated with a decrease in urine flow and in excreted Na^+ and K^+ (Bradley et al., 1954; Lowrance et al., 1956). In fact, reductions in renal clearance induced by apnea are so profound that, in Na^+ , K^+ - and water-loaded harbor seals, an increase in electrolyte and water retention was observed despite the body's need to eliminate these excess cations and fluid (Bradley et al., 1954). Lowrance et al. (Lowrance et al., 1956) also demonstrated that apnea and anoxia have similar effects on renal hemodynamics in the harbor seal. However, in harbor seals trained to dive voluntarily and continuously without intermittent periods of breathing (the two main differences between the earlier and most recent studies), apnea resulted in complete cessation of urine flow and GFR, which was attributed to an arterial constrictor response, within the first minute of the dive (Murdaugh et al., 1961b). In contrast, GFR did not change from resting values during natural aerobic dives in Weddell seals (*Leptonychotes weddellii*), although GFR decreased by over 90% during dives greater than the animal's aerobic dive limit (ADL), which was longer than 9 min (Davis et al., 1983). This decrease in GFR corresponds well with the 91% decrease in blood flow (ml min^{-1}) to the kidneys of Weddell seals measured 8–12 min into their simulated dive (Zapol, 1979), which is within the range of time exceeding the ADL of this species (Davis et al., 1983). Therefore, the reduction in GFR appears to be the consequence of regional shunting of blood flow to the kidneys. Under non-diving conditions, the kidneys are energetically expensive (they have a high rate of oxygen consumption) and receive a large volume of blood for filtration purposes. Therefore, shunting blood away from the kidneys during dives longer than the animal's ADL provides an adaptive advantage from the perspective of decreasing oxygen consumption by the kidneys and, thus, increasing the delivery of oxygenated blood to other tissues.

Water immersion

The immersion of humans in water results in a diuresis associated with an increase in thoracic and mean arterial pressures (Epstein, 1992). This connection between the cardiovascular system and the kidney was termed the Henry–Gauer reflex after the two researchers who demonstrated that arterial distention in the dog resulted in an almost immediate diuresis (Gauer and Henry, 1976). For pinnipeds, which spend more than 75% of their time submerged in water, this reflex would be disadvantageous because of increased urinary water loss. The pressure effects of water immersion were simulated in harbor seals by continuous negative-pressure breathing, but no change in urine output was induced (Murdaugh et al., 1961a). The lack of a diuretic response to negative-pressure breathing suggests that

a Henry–Gauer reflex is not present in seals, which would be advantageous for a mammal living primarily in water.

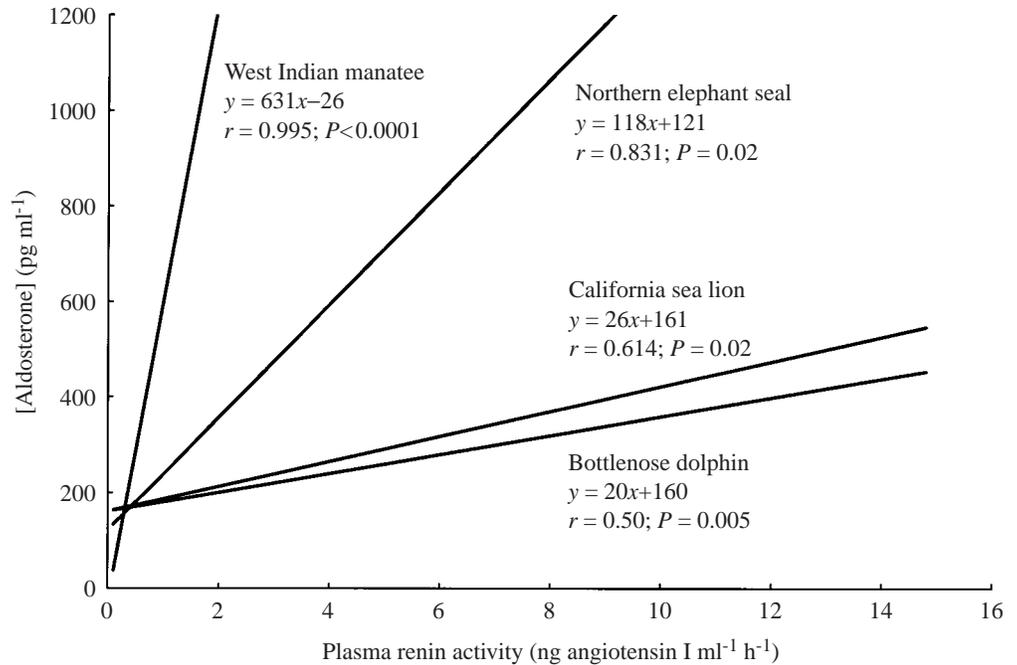
Hormonal regulation

In mammals, the primary hormones responsible for osmoregulation are angiotensin (angiotensin I, II or III), atrial natriuretic peptide (ANP), aldosterone and vasopressin (AVP). Renin converts angiotensinogen to angiotensin I, which is quickly converted to angiotensin II (the most potent angiotensin) and finally to angiotensin III by angiotensin-converting enzyme (Ichikawa and Harris, 1991). Subsequently, angiotensin II stimulates the release of aldosterone from the adrenal gland, which in turn induces the resorption of Na^+ in the distal tubule of the nephron, resulting in a decrease in excreted Na^+ (Funder, 1993). Atrial distention, induced by increased cardiac pressure (volume), is the primary mechanism for release of ANP (Stanton, 1991). The actions of ANP oppose those of angiotensin II and aldosterone by inhibiting the synthesis and release of renin, thereby resulting in an increase in excreted Na^+ (Stanton, 1991). Aldosterone and angiotensin II also have water-retention qualities (Ichikawa and Harris, 1991; Funder, 1993), but the most potent anti-diuretic agent is vasopressin (Wade et al., 1982), which stimulates the synthesis of water channels (aquaporins) in the collecting duct (Nadler, 1998).

Circulating concentrations of the four primary hormones involved in osmoregulation have been reported for a variety of pinnipeds (St. Aubin and Geraci, 1986; Zenteno-Savin and Castellini, 1998a; Ortiz et al., 2000). Although most seals live in salt water, they do not drink sea water, and captive animals may be susceptible to hyponatremia (Geraci, 1972; St. Aubin and Geraci, 1986). Hyponatremia appears to be reconciled *via* the pituitary–adrenal axis (St. Aubin and Geraci, 1986). The increase in fractional clearance of Na^+ following infusion of hyperosmotic saline indicates that tubular Na^+ resorption is reduced (Hong et al., 1982), suggesting that the level of aldosterone is decreased. However, a negative correlation between excreted aldosterone and excreted Na^+ levels was not observed, prompting Hong et al. (Hong et al., 1982) to question the role of the renin–aldosterone axis in seals. However, evidence for a functional renin–angiotensin–aldosterone system has been obtained in elephant seals (Ortiz et al., 2000). Positive correlations between plasma renin activity (an indicator of angiotensin generation) and aldosterone levels in California sea lions (Malvin et al., 1978) and fasting northern elephant seal pups (Ortiz et al., 2000) (Fig. 2) suggest that, under normonatremic conditions, electrolyte balance is regulated *via* the renin–angiotensin–aldosterone system (RAAS). A comparison of the slopes of the regressions between plasma renin activity and aldosterone level for these two pinnipeds indicates that the sensitivity of the system is increased when Na^+ availability is reduced, such as in the case of fasting elephant seals (Fig. 2).

Although not conclusive, a number of studies provide compelling evidence to suggest that tubular water resorption is mediated *via* AVP in seals, as in terrestrial mammals (Wade et

Fig. 2. Correlation between plasma renin activity and aldosterone levels in four species of marine mammal, bottlenose dolphin (*Tursiops truncatus*), California sea lion (*Zalophus californianus*), northern elephant seal (*Mirounga angustirostris*) and West Indian manatee (*Trichechus manatus*). Data for dolphins ($N=3$) and sea lions ($N=4$) are individual points extrapolated from Figs 2 and 3 in Malvin et al. (Malvin et al., 1978). Data for fasting, postweaned elephant seal pups ($N=15$) are means extrapolated from Fig. 1 of Ortiz et al. (Ortiz et al., 2000). Data for West Indian manatees ($N=4$) held in fresh water are means extrapolated from Fig. 2 of Ortiz et al. (Ortiz et al., 1998). Regressions were considered significant at $P<0.05$.



al., 1982). The intravenous infusion of pitressin (synthetic AVP) in a water-loaded harbor seal resulted in an immediate decrease in urine flow rate together with concomitant increases in urinary electrolyte concentrations (Bradley et al., 1954). Pitressin infusions induced an increase in urine osmolality and an increase in osmotic clearance (C_{osm}) that was greater than urine volume, suggesting that resorption of free water was increased (or clearance of free water was reduced) (Page et al., 1954). Under force-fasted conditions, Baikal and ringed seals exhibited an increase in excreted AVP associated with a concomitant decrease in urine flow rate and increase in urine osmolality (Hong et al., 1982). Hong et al. (Hong et al., 1982) also demonstrated a positive and significant correlation between urine osmolality and excreted AVP, further suggesting that the observed increase in urine osmolality was attributable to an increase in tubular water resorption *via* AVP stimulation. In grey seals, force-fasted conditions induced an increase in urine osmolality in conjunction with an increase in plasma osmolality and AVP (Skog and Folkow, 1994). However, naturally fasting, postweaned elephant seal pups demonstrated an increase in urine osmolality despite a decrease in plasma AVP (Ortiz et al., 1996). These latter data were refuted recently, using a much larger sample of fasting pups, to demonstrate that plasma AVP concentrations are constant and relatively low with no change in plasma osmolality (Ortiz et al., 2000).

As described above, diving has profound effects on blood flow to the kidneys and on glomerular filtration, resulting in a decrease in renal activity, which could alter the response of vasoactive hormones (e.g. angiotensin II, AVP, ANP). In fact, voluntary bouts of sleep apnea in elephant and Weddell seal pups resulted in a reduction in heart rate associated with a decrease in levels of angiotensin II and AVP (vasoconstrictors)

and an increase in the level of ANP (a vasoconstrictor-inhibitor) (Zenteno-Savin and Castellini, 1998b). Zenteno-Savin and Castellini (1998b) attributed the increase in ANP to an increase in cardiac pressure, which is a known stimulus for ANP release (Stanton, 1991). The changes in levels of vasoactive hormones observed during sleep apnea are also likely to occur during breath-hold diving on the basis of the available data on renal blood flow during diving and the direction of change in levels of vasoactive hormones during sleep apnea.

Collectively, it would appear that water and electrolyte resorption are mediated by AVP and the RAAS, respectively, in pinnipeds, as they are in terrestrial mammals. During hyponatremic conditions, mediation *via* the pituitary–adrenal axis may also be required to replete salt concentrations. Apnea increases ANP levels and decreases angiotensin II and AVP levels, suggesting that these vasoactive hormones are also influenced by diving and may help regulate blood pressure and flow to the kidneys.

Dolphins and whales

For logistical reasons, very little information on the osmoregulation of cetaceans exists; the majority of data are confined to dolphins and porpoises. Studies prior to 1940 consisted primarily of measurements of freezing point depression (osmolality) and electrolyte concentrations in different biological fluids from various species (Fetcher, 1939).

Drinking, fasting and feeding

Probably the first experiments on cetacean osmoregulation consisted of the oral administration of hypertonic NaCl to a bottlenose dolphin (Fetcher and Fletcher, 1942). The results of

these experiments suggested that dolphins do not drink sea water (Fetcher and Fletcher, 1942). However, more recently, isotopic dilution techniques were incorporated to address the question of water flux and consumption in four species of delphinid, common dolphin (*Delphinus delphis*) (Hui, 1981), Pacific white-sided dolphin (*Lagenorhynchus obliquidens*), Pacific bottlenose dolphin (*Tursiops gilli*) and pilot whale (*Globicephala scammoni*) (Telfer et al., 1970). These studies demonstrated that, under fasting conditions, these species consumed sea water at rates ranging between 4.5 and 13 ml kg⁻¹ day⁻¹ (Telfer et al., 1970; Hui, 1981).

Telfer et al. (Telfer et al., 1970) concluded that absorption of water through the skin was unlikely. However, later studies reported that the skin is a major avenue of water flux that may account for as much as 70% of total flux in fasting dolphins (Hui, 1981). Water flux across the skin was also reported to be dependent on the osmotic gradient of the environment, suggesting that delphinids with access to a hypo-osmotic habitat could experience a net gain in fresh water (Andersen and Nielsen, 1983).

Contrary to the water-conservation mechanism of concentrating urine discussed above for pinnipeds, fasting cetaceans have been shown to produce a dilute urine (Bentley, 1963; Telfer et al., 1970; Hui, 1981). Telfer et al. (Telfer et al., 1970) suggested that the animals might become acutely dehydrated, which would serve as a stimulus to increase metabolic water production (*via* fat oxidation), resulting in the increased excretion of free water. Hui (Hui, 1981) demonstrated that metabolic water was sufficient to preclude the necessity for drinking sea water and for excreting a concentrated urine to maintain water balance. This idea is further supported by the observation that cetaceans do not consistently exhibit the urine-concentrating ability of pinnipeds (Table 1). Therefore, the quantification of seawater drinking in fasting dolphins (Telfer et al., 1970; Hui, 1981) is intriguing and poses the question as to why they drink.

As in pinnipeds, GFR, RPF and urine flow rate in dolphins increased after feeding, resulting in an increase in electrolyte excretion (Malvin and Rayner, 1968; Ridgway, 1972). Because the dolphins were fed a high-protein diet of raw fish, it is likely that the observed increases in GFR and RPF were induced by increased nitrogen-loading on the kidney, as in the seal (Hiatt and Hiatt, 1942).

Solute and water loading

Administration of hyperosmotic saline or fresh water have all been achieved by stomach tube in dolphins (Fetcher and Fletcher, 1942; Malvin and Rayner, 1968; Ridgway, 1972). A hypertonic saline load increased urine flow (Fetcher and Fletcher, 1942; Ridgway, 1972), while freshwater loading (2.5% of body mass) did not induce diuresis, although an increase in urine osmolality was observed 2h after the administration (Malvin and Rayner, 1968). Malvin and Rayner (1968) concluded, on the basis of their data and that of Fletcher and Fletcher (Fletcher and Fletcher, 1942), that the increased diuresis following a hypertonic infusion was the result of

extracellular fluid expansion. However, Ridgway (Ridgway, 1972) repeated the tests of Malvin and Rayner (Malvin and Rayner, 1968) using a different species of dolphin (*Tursiops truncatus* and *T. gilli*, respectively) and demonstrated a diuresis following freshwater loading after 2h. Although Ridgway (Ridgway, 1972) mentioned that a species difference may have accounted for the discrepancy in the results, the two animals were of similar body mass (187 *versus* 160 kg) and received the same dose (Table 4). Probably the most glaring discrepancy between the two studies was the urine collection time: 2h in one study (Malvin and Rayner, 1968) and 24h in the other (Ridgway, 1972). Therefore, a time-delayed response may have contributed more to the difference between the two studies than a species-specific effect.

Other points of interest

As in pinnipeds, the secretion of creatinine from the renal tubules into the filtrate was not observed in dolphins (Malvin and Rayner, 1968), suggesting that the use of creatinine clearance provides a valid estimate of GFR in cetaceans as well. Respiratory water loss was determined to be low in dolphins, as in pinnipeds, ranging between 30 and 77% of that expected for a terrestrial mammal of similar body mass (Coulombe et al., 1965) and suggesting that this may be a significant mechanism for maintaining water balance in cetaceans.

Hormonal regulation

Probably the first published account of a bioassay using extracted cetacean tissues was that of Eichelberger et al. (Eichelberger et al., 1940). Extracted renin from the kidney of a bottlenose dolphin was injected into dogs at two doses, 4 ml and 2 ml. In the initial trial, 4 ml of extract induced an immediate 48% increase in blood pressure, which remained 38% higher than control pressure for 10 min. In a separate trial, an initial 2 ml dose of extract induced a 21% increase in blood pressure, but it was not sustained. A second 2 ml dose resulted in a 27% increase in blood pressure, which was also not sustained. However, the two doses collectively were able to induce a 9% increase in blood pressure that was sustained. A third dose of 2 ml produced a 20% increase in blood pressure that sustained an additional 4% increase in blood pressure. These experiments demonstrated (i) that renin in dolphins (and probably all cetaceans) causes a vascular pressor effect as in other mammals, (ii) that the pressor effect is dose-dependent, and (iii) that the response is graded until a threshold is reached that results in a sustained elevation in blood pressure. Unfortunately, the concentrations of the doses were not reported. However, sufficient information on the extract was provided to enable the experiments to be repeated using known concentrations of the doses and thus to shed light on the sensitivity and kinetics of the renin-induced increase in blood pressure.

Concentrations of circulating renin were reported 27 years later (Malvin and Vander, 1967). Eleven years later, Malvin et al. (Malvin et al., 1978) demonstrated a positive correlation

between plasma renin activity and aldosterone content, suggesting that the RAAS is present and active in bottlenose dolphins. The sensitivity of the RAAS appears to be the same as that of California sea lion, as indicated by the similarity in slopes of their correlation regressions (Fig. 2). Positive correlations among adrenal steroid levels (aldosterone, cortisol and corticosterone) have also been exhibited in bottlenose dolphins, suggesting the presence of an active pituitary–adrenal axis (Ortiz and Worthy, 2000).

The only other known bioassay of cetacean tissue was that of plasma-extracted AVP on water retention in water-loaded rats by measuring the electrical conductivity of the urine (Malvin et al., 1971). In most of the dolphin samples, AVP was not detected by a bioassay. For those samples that showed detectable amounts of AVP, concentrations were very low, as were the amounts in pituitary extracts. Also, a correlation between urine flow rate and plasma AVP concentrations in fasting dolphins was not observed, leading Malvin et al. (Malvin et al., 1971) to contend that AVP does not significantly regulate urine volume and, thus, water retention in these animals. However, significantly greater concentrations of AVP have been measured recently in free-ranging dolphins, which may be the result of advances in assay techniques for hormone measurements (Ortiz and Worthy, 2000).

Manatees and dugongs

Species of the Order Sirenia provide an intriguing model for comparative studies of osmoregulation among marine mammals because members of this group are strictly herbivorous (Best, 1981; Bengtson, 1983) and inhabit the full range of salinity, from the freshwater Amazonian manatee to the marine dugong. Among sirenians, the West Indian manatee may provide the best model for study because of its ability to move between fresh water and a marine environment with no deleterious effects (Ortiz et al., 1998). The vast majority of data that exist on osmoregulation in sirenians were obtained from studies on the West Indian manatee.

Drinking

The manatee's ability to concentrate its urine led to speculation that it may consume sea water to maintain fluid homeostasis (Irvine et al., 1980). However, water turnover studies using deuterium oxide revealed that manatees do not consume sea water voluntarily and that, during periods of food deprivation, their water needs are met from metabolic water production, as in pinnipeds and cetaceans (Ortiz et al., 1999) (Table 3).

Plasma data

Water flux studies have demonstrated that consumption of fresh water can be high, leading to lower osmotic and ionic concentrations (Ortiz et al., 1998; Ortiz et al., 1999). Plasma osmolality and electrolyte concentrations in wild freshwater manatees (Irvine et al., 1980; Medway et al., 1982; Ortiz et al., 1998) are similar to those of wild and captive animals in salt

water (Ortiz et al., 1998), suggesting that wild animals in fresh water have occasional access to salts either by temporarily residing in a marine environment or by consuming aquatic vegetation with a sufficient salt content. Therefore, captive manatees held permanently in fresh water may be susceptible to hyponatremia, as are captive seals in a salt-depleted environment (Geraci, 1972; St. Aubin and Geraci, 1986).

To date, no study of renal hemodynamics in any sirenian has been conducted. If renal blood flow and filtration rate are associated with dietary nitrogen levels in manatees, as they are suspected to be in seals (Hiatt and Hiatt, 1942) and possibly in dolphins, then RPF and GFR may be relatively low because of the low protein content of their diet (Best, 1981; Bengtson, 1983) and milk (Pervaiz and Brew, 1986). Alternatively, because freshwater manatees consume large volumes of water, RPF and GFR may be elevated in these animals as a result of a hypervolemic-induced increase in blood pressure.

Hormonal regulation

Unfortunately, the endocrinology of water and electrolyte balance has received little attention, with only one published account to date (Ortiz et al., 1998). As in pinnipeds and cetaceans, the RAAS is present and appears to regulate Na⁺ balance in manatees (Ortiz et al., 1998). Freshwater manatees exposed to salt water for 4 days exhibited a significant decrease in the response of the RAAS and subsequent increase upon returning to fresh water. The sensitivity of the RAAS in manatees is much greater than in pinnipeds and dolphins (Fig. 2), which would be expected since manatees are commonly found in Na⁺-depleted environments (Best, 1981) and do not drink sea water (Ortiz et al., 1999). Also, oral intubation resulted in a neuroendocrine stress response, producing an increase in the response of RAAS (Ortiz et al., 1998). Increased plasma osmolality appears to stimulate AVP release in manatees (Ortiz et al., 1998), as in terrestrial mammals (Wade et al., 1982), but the antidiuretic actions of AVP in manatees have yet to be elucidated.

Otters

Otters have representative species in both a strictly freshwater habitat, the American river otter (*Lutra canadensis*) (Hoover and Tyler, 1986), and a strictly marine habitat, the sea otter (*Enhydra lutris*) (Costa, 1982). Unfortunately, very little comparative data on their osmoregulatory capabilities exists.

Similar to the kidneys of pinnipeds and cetaceans, otters also possess a lobulate kidney (Hoover and Tyler, 1986) but, with respect to body mass, it is larger than that of other marine mammals (Costa, 1982). Sea otters possess a slightly greater urine-concentrating ability than river otters, with the reported maximum urine osmolality in the sea otter being approximately 30% greater (Table 1). The river otter, like the Baikal seal and West Indian manatee, has retained the ability to concentrate its urine despite adapting to a freshwater habitat.

Plasma Cl⁻ concentrations in the two otter species are similar, although blood urea nitrogen (BUN) is higher in the

river otter. Not surprisingly, urinary Na⁺ and Cl⁻ levels are approximately 6.5- and 10-fold greater in the sea otter (Table 2). Because sea otters can excrete Na⁺ and Cl⁻ in greater concentrations than are found in their environment, they possess the ability to actively consume sea water and to gain free water (Costa, 1982). Sea otters are the only marine mammals reported actively to consume sea water and they do so as a means of eliminating urea-nitrogen load (Costa, 1982). Again, the high-protein diet of otters suggests that changes in GFR and RPF may be associated with feeding and fasting.

The absence of hormonal data with respect to water and electrolyte balance in otters is surprising.

Future directions

Each of the taxonomic groups of marine mammals has at least one member that inhabits solely a freshwater environment, but only studies on the renal function of seals found naturally in fresh water have been conducted (Hong et al., 1982). Comparative renal studies of representative species in each of the major taxonomic groups of marine mammals would provide instructive information about the evolution of osmoregulatory functions in marine-adapted mammals. At present, it appears that freshwater species do not exhibit a diminished renal capacity, indicating that they have not lost the osmoregulatory functions of their marine counterparts. Although a large number of studies involving feeding, fasting and infusions have provided a wealth of information on their effects on renal function, the underlying mechanisms regulating the excretion and resorption of water and solutes remain unexamined. Therefore, studies incorporating a hormonal component would be of interest. For example, the involvement of ANP in Na⁺ excretion has yet to be examined in any marine mammal. Also, the anti-diuretic function of AVP in marine mammals remains inconclusive and warrants further investigations. A comparison of osmoregulatory hormones between river and sea otters would be of interest. The present review has indicated a number of important areas that warrant further or initial examination to elucidate osmoregulation in marine mammals.

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