THE OSMOTIC AND IONIC REGULATORY CAPACITIES OF THE
KIDNEY OF THE HARBOR SEAL, PHOCA VITULINA

by

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ABSTRACT

The mechanisms of osmotic and ionic regulation in marine mammals are of interest because of the apparent lack of "fresh" water in their environment. Previous investigation on the harbor seal, (Phoca vitulina, L.), generally indicated that the seal can obtain all the water it requires from its food. However, some dispute still exists as to whether the seal may ingest sea water along with its food and conserve water by concentrating ions and excreting them with a net water gain.

The effects of a 16-hour period with no fluids and also of intubation with varying amounts of distilled water and varying amounts and concentrations of sea water were determined. The concentrations of sodium, chloride and potassium ions as well as the osmotic pressures of plasma and urine were measured for the periods before and after intubation.

The results of this study are discussed with respect to published data and proposed mechanisms of osmotic and ionic regulation by the kidney. The findings indicate, as suggested by others, that the seal does not gain any substantial amount of water from sea water ingestion.

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INTRODUCTION

The physiological adaptations of marine mammals to their environment have been of interest to biologists for some time and have been recently reviewed by Harrison and Tomlinson (1961), Lilly (1964), Scholander (1964) and Andersen (1966). The question of osmotic and ionic regulation has been of particular interest because of the apparent lack of "fresh" water available to these animals.

With reference to water balance and osmotic regulatory mechanisms, the most extensively studied marine mammal has been the harbor seal, phoca vitulina. This seal has been found considerable distances up rivers and in estuaries although the vast majority lives entirely in a marine environment (Fisher, 1952 and Scheffer, 1958). Those harbor seals in fresh and estuarine habitats have an abundant supply of fresh water. However, to renew its body water, a seal which lives in sea water would have to migrate to river mouths periodically to drink fresh water, drink sea water and excrete a hypertonic urine, or obtain water from the metabolic breakdown of its food. Periodic migration to fresh water seems unlikely in view of observations by the author of some seals surviving without fresh water on islands in a marine setting, for example Pribilof Islands.

Irving, et al. (1935) examined the chloride content of urine and fecal matter from twenty young harbor seals kept in sea water and determined that the chloride content was comparable to that of terrestrial mammals. They

concluded that the seal did not drink sea water and that the seal's kidney was not unique in its ability to excrete salt. From calculations involving the amount of water available from metabolism of herring and the possible physiological uses of this water, they concluded that the harbor seal could manage adequately on the water from ingested fish.

Smith (1936) examined the inorganic composition of the urine and the composition of salts of rectal washings of one captive harbor seal. Smith concluded that the seal did not swallow any considerable quantity of sea water. He assumed, on the basis of Irving's work and his own results, that body water and water for urine formation are derived from water in the food and from metabolism. Albrecht (1950) intubated harbor seals with sea water and after observing the reactions of the animals, vomiting and diarrhea, concluded that they do not drink sea water. She also dehydrated the animals and observed that the seals could not be induced to drink sea water.

Fresh water drinking has been reported by Irving, et al. (1935) and Albrecht (1950) and has been observed in newly arrived seals of the U.B.C. colony. In all cases, dehydration of the seals had occurred prior to drinking. However, these seals have never been observed to drink sea water. If it can be assumed that the seal does not drink sea water, the question of how it conserved its supply of water should be considered. Irving, et al. (1935) calculated that of the 1100 grams of water yielded by 1250 grams of herring, 300

grams would be used for perspiration, respiration and the formation of feces, and the remaining 800 grams would be available for urine formation. Although the seal does have sweat glands, Irving, et al. (1935) assumed that evaporated water from perspiration is not necessary for thermal regulation since the animal resides in a cool aquatic habitat. Smith (1936), Hiatt and Hiatt (1942) and Page, et al. (1954) observed an increase in urine volume in the seal after a meal of herring and a decrease in urine flow between feedings. The Hiatts suggested that the kidney activity increases when there is water to spare for excretion and decreases between meals in order to conserve water. The Hiatts further suggested that post-feeding renal vasodilation occurs only when water is available, vasoconstriction and the associated reduction in glomerular filtration limiting water excretion at other times. They postulated that it was this reduction of blood flow in the kidneys, and not the change in the number of active glomeruli, that was responsible for the reduced urine output.

Diving and its associated reduction of blood flow to various organs has been shown to play an important role in conserving body water. Irving, et al. (1935) initially demonstrated that during diving there was a decrease in peripheral blood flow in the seal. Furthermore, Bradley and Bing (1942) found that during diving there was marked vasoconstriction of arteries leading to the kidneys with almost complete cessation of glomerular filtration and urine

formation. They concluded that the intra-renal vasoconstrictive response to apnea appears to involve both afferent and efferent arterioles in a relatively uniform manner. Bradley, et al. (1954) observed that sodium, potassium and water excretion decreased greatly with reduction in filtration during apnea. Lowrance, et al. (1956) compared the effects of anoxia and apnea in the harbor seal and concluded that they have comparable effects on renal activity. During diving there is a stoppage of breathing and consequently no water lost by evaporation from the lungs.

A histological examination of the nephrons of the kidney of the harbor seal has not been attempted. However, Sperber (1944) has examined nephrons of two closely related pinnipeds, Phoca barbata and Phoca hispida. The length of the loops of Henle and medullary thickness in these two seals were comparable to those of terrestrial carnivores, thereby supporting the physiological data of Irving, et al. (1935) that these aquatic animals can form a hyperosmotic urine, but not as concentrated as would be expected if they drank sea water.

Several investigators have answered, in part, the question whether the seal can conserve water from sea water by forming a hyperosmotic urine. Albrecht (1950) recorded the highest urine chloride ion concentration after intubating sea water. From her graph the value would be approximately 500 milliequivalents per liter (mEq./l.). The highest urine sodium and potassium values reported in the literature are

480 and 370 mEq.l., respectively (Bradley, et al, 1954). These values indicate that the seal cannot excrete all of these ions in urine at concentrations higher than those of open sea water (chloride: 548.3; sodium: 470.2; potassium: 9.9 (Barnes, 1954)).

In the harbor seal neither the osmotic and ionic regulatory capacities of the kidney, nor the excretory patterns following ingestion of sea water have been fully examined. In this study the effects of dehydration of the seal and intubation of distilled water and of sea water in varying volumes and concentrations were examined and the following points considered: 1. the urine volumes and the concentrations of sodium, chloride and potassium in the plasma and urine over a period of thirteen hours after intubating distilled water and sea water. 2. the maximum urine osmotic pressures and the maximum sodium, chloride and potassium concentrations that the kidney of the harbor seal is capable of producing. 3. the source of water, either ingested or produced from body-water reserves, which is used to eliminate the ions ingested.

Consideration of these problems will help determine whether the kidney has any special osmotic or ionic regulatory adaptations that enable the seal to survive in the marine environment.

MATERIALS AND METHODS

ANIMALS: Two yearling female harbor seals captured off Northern Vancouver Island were studied over a one year period. The seals were kept in wood-stave tanks, nine feet in diameter, which contained two feet of running fresh water. Over the test period they were each fed about five pounds of thawed herring daily and maintained their weights at 95 ± 8 pounds.

SAMPLING PROCEDURE: To ensure that the seals were in a post-absorptive state, they were starved for 36 hours prior to an experiment. One hour after removing the animal from its tank and restraining it, the first urine sample was taken and one-half hour later the first blood sample was taken. Over the next 16 hours, the presence of fluid around the eyes, mouth and nostrils were recorded hourly. The room temperature was maintained at $67 \pm 2^{\circ}\text{F}$.

The following fluids were intubated in individual experiments two hours after the initial urine collection: 500 ml. and 1000 ml. of distilled water; 500 ml., 750 ml. and 1000 ml. of sea water; and 750 ml. of sea water which had been evaporated to give 1.5 times the normal sea water concentrations of the ions to be studied ($1\frac{1}{2} \times \text{S.W.}$). In a control experiment, to determine the effects of dehydration and stress during the 16 hour collection period, the animal was restrained and sampled in the above manner, but no fluid was intubated. The control experiment was done once for each animal and was the first test carried out.

- a. Urine Collection: The hind flippers of the seal were strapped to the restraining board. A plexiglass speculum (3.5" in length and 1.5" in diameter) was placed into the urogenital vestibule as far as the urinary papilla. A sterile X-ray catheter was then inserted into the bladder and the urine was collected in a sterile polyethylene bag (Whirlpac Co.). After recording the volume, the urine was pipetted into containers for analysis. Between collections the flippers were free to move. A glass dish was placed to catch any urine or fecal material voided between sampling times.
- b. Blood Collection: The hair above two lumbar vertebrae was removed and the skin scrubbed with an antiseptic (Phisoex) and with 70% ethanol. In this region a local anesthetic (10 ml. of 1% xylocaine) was injected intramuscularly. An 18-gauge thin-walled needle with fitted stylet was inserted between two neural spines of the vertebrae into the intravertebral-epidural vein. The stylet was replaced with a sterile polyethylene canula (Intramedic PE 50) and then the needle was removed leaving the canula in place. To prevent blood from clotting in the canula, it was filled with a 0.1% sodium heparin solution. Blood was withdrawn in a heparinized 10 ml. disposable syringe from which the liquid heparin had been forcibly removed. A 21-gauge needle was used to connect the syringe to the canula. After each

10 ml. blood sample was collected, the canula was filled with heparin. The whole blood was centrifuged for 25 minutes at 3500rpm and the plasma pipetted into containers for analysis.

ANALYTICAL PROCEDURES:

- a. Osmotic Pressure: Two milliliter aliquots of each urine and plasma sample were pipetted into osmometer tubes. The number of milliosmoles was determined on a Fiske Osmometer (Model G-62) which had been calibrated using prepared sodium chloride standards so that direct readings ranging from 0 to 3000 milliosmoles could be taken. The samples were analysed within four hours after the completion of each experiment.
- b. Chloride Ion Determination: The chloride ion concentration in the urine and plasma samples was determined using a Buchler-Cotlove chloridometer. Solutions were prepared by combining 0.1 ml. of sample with 4 ml. of a nitric-acetic reagent (0.1 N HNO_3 and 10% glacial acetic acid). Standards were prepared for both HIGH and LOW rates of titration by adding 0.1 ml. of 104 mEq./l. and 0.1 ml. of 1 mEq./l. sodium chloride solution, respectively, to 4 ml. of the nitric-acetic acid reagent. Method blanks of 4 ml. of nitric-acetic acid reagent were also prepared. Before titration 0.2 ml. of gelatin pH-indicator solution was added to each vial.
- c. Sodium Ion Determination: Samples of urine and plasma were prepared by diluting 1 μl . of sample in 5 ml. of distilled, demineralized water in polyethylene vials

(Nalgene Co.). Prepared samples were frozen and stored at -10°C . until the sodium ion concentration could be determined. Sodium chloride standards were prepared by diluting 1 μl . of stock solutions in 5 ml. of distilled, demineralized water and were used to calibrate the Unicam flame photometer (Model SP900) and to obtain a standard curve. Samples were analyzed on the flame photometer and the sodium ion concentration (mEq./l.) was determined from the standard curve.

d. Potassium Ion Determination: Samples of urine and plasma were prepared by diluting 50 μl . of sample into 5 ml. of distilled, demineralized water. The prepared samples were frozen and stored at -10°C . until the concentrations could be determined. The Unicam flame photometer was calibrated using 50 μl . of KCl standards diluted in 5 ml. of water. Potassium ion concentrations in the samples were determined from the standard curve which ranged from 0 to 275 mEq./l. "Lab-trol" (Dade Reagents Inc.), a control serum in liquid form containing known concentrations of substances found in the blood, was used in all chloride, sodium and potassium ion determinations as a standard.

RESULTS

Initially, three seals were chosen for study, however, one animal was unable to take the stress of the experimental conditions and gave very erratic results or became ill during the experiments. Thus, in the final analysis, only two seals could be used. Although the qualitative responses of the two seals are similar, there are obvious quantitative differences. The changes in plasma osmolality prior to and following intubation of fluids are shown in Figures 1a-1g. Each of the intubation experiments was compared to the control in which no fluid was given. It can be noted that the preintubation values varied between 318 and 330 mOsm./l. for seal #1 and between 318 and 343 mOsm./l. for seal #2. Intubation of 500 ml. of distilled water and 500, 750 and 1000 ml. of sea water does not appear to have any great effect on the osmolality of the plasma if one considers the changes from the preintubation values and the changes observed in the control. However, 1000 ml. of distilled water decreased the osmolality of the plasma to as low as 305 mOsm./l. (Seal #1) and 308 mOsm./l. (Seal #2). With 750 ml. of $1\frac{1}{2}$ x sea water the osmolality of the plasma increased for 3 - 4 hours after intubation and then returned gradually to the preintubation values.

In analysing the urine osmolality (Figure 2a-2g), it can be seen that during the control tests, Seal #2 had a much higher osmotic pressure than Seal #1. After distilled water ingestion, the seals responded similarly with the

FIGURE 1: Changes in plasma osmolality (mOsm./l.).

Intubation at 0 time.

- a. Control
- b. 500 ml. distilled water
- c. 1000 ml. distilled water
- d. 500 ml. sea water
- e. 750 ml. sea water
- f. 1000 ml. sea water
- g. 750 ml. $1\frac{1}{2}$ x sea water

Seal #1 —————

Seal #2 - - - - -

FIGURE 2: Changes in urine osmolality (mOsm./l.) and specific gravity. Intubation at 0 time.

- a. Control
- b. 500 ml. distilled water
- c. 1000 ml. distilled water

Seal #1 ———

Seal #2 -----

Specific gravity

Time (hours)

FIGURE 2 (b): Changes in urine osmolality (mOsm./l.)
and specific gravity. Intubation at
0 time.

- d. 500 ml. sea water
- e. 750 ml. sea water
- f. 1000 ml. sea water
- g. 750 ml. $1\frac{1}{2}$ x sea water

Seal #1 _____

Seal #2 - - - - -

Time (hours)

osmolality decreasing within one hour following intubation. In both the tests with 500 ml. and 1000 ml. of distilled water, the osmolality reached the minimum value 5 hours after intubation and then started to increase immediately in the former case and after another 5 hours in the latter. One hour following the ingestion of sea water, the two seals gave different results for the 500 ml. test. With Seal #1 there was no overall change in osmolality for 500 ml. of sea water, Seal #2 increased its urine osmolality throughout the experiment. However, following intubation of 750 ml. and 1000 ml. of sea water, and 750 ml. of $1\frac{1}{2}$ x sea water experiments the results were qualitatively similar. For the 750 and 1000 ml. sea water tests there is an overall increase in osmolality above the preintubation value with Seal #2 increasing earlier and to a higher value. In the $1\frac{1}{2}$ x sea water tests there was less overall change in osmolality (Seal #1 increased 100 and Seal #2 increased 200 mOsm./l.), whereas in the 750 and 1000 ml. sea water tests there was an increase of at least 150 mOsm./l. for Seal #1 and 600-700 mOsm./l. for Seal #2. Although there were individual variations, the results indicated no large overall change in plasma osmolality.

Figure 2a-2g also show the relationship between specific gravity of the urine at different times and the osmolality. In the control and distilled water experiments, the trends in these two values are very similar. With sea water tests, however, there is a drop in the specific gravity within the second hour after intubation, but no corresponding drop in

the osmolality. By the end of the sampling time, the specific gravity had increased and the urine of Seal #2 had the higher specific gravity and also the higher osmotic pressure. The specific gravity values as a reflection of total solids in the urine may help account for the osmolality changes observed.

The chloride concentrations in the plasma samples were determined and are presented in Figure 3a-3g. In comparing the patterns of changes in concentrations to that of the control, it can be seen that no obvious differences occur following distilled water intubation. However, with ingestion of sea water, the chloride ion concentration appears to be increased over the preintubation values in both seals.

Changes in plasma sodium concentrations are shown in Figure 4a-4g. Again, the preintubation values vary (between 152 and 172 mEq./l.). There appear to be no measurable effects on the sodium concentrations following intubation with distilled water. Further sampling is necessary to determine if the peaks in the concentration after sea water ingestion are significant and repeatable. Figures 4e-4g indicate that the sodium ion concentration fluctuates as much as it does in the control and distilled water experiments.

Plasma potassium concentrations were measured, but did not change greatly in any of the tests, varying between 4.3 and 5.0 mEq./l. No obvious pattern of variation was detected.

The more interesting results were obtained with analysis of urine volume and the concentration of sodium, chloride and potassium in the urine following intubation.

FIGURE 3: Changes in plasma chloride concentration
(mEq./l.) with time. Intubation at 0 time.

- a. Control
- b. 500 ml. distilled water
- c. 1000 ml. distilled water
- d. 500 ml. sea water
- e. 750 ml. sea water
- f. 1000 ml. sea water
- g. 750 ml. $1\frac{1}{2}$ x sea water

Seal #1 ———

Seal #2 -----

FIGURE 4: Changes in plasma sodium concentration
(mEq./l.) with time. Intubation at 0 time.

- a. Control
- b. 500 ml. distilled water
- c. 1000 ml. distilled water
- d. 500 ml. sea water
- e. 750 ml. sea water
- f. 1000 ml. sea water
- g. 750 ml. $1\frac{1}{2}$ x sea water

Seal #1 ———

Seal #2 -----

The urine volumes are presented in Figure 5a-5g. The average rate of volume output (ml./hour) in the control, in which no fluid was intubated, was 14.8 for Seal #1 and 13.8 for Seal #2. Intubation of 500 ml. of distilled water increased the average excretion rate to 26.9 and 19.1 ml./hour, and 1000 ml. increased it to 38.1 and 27.7 ml./hour in seals #1 and #2, respectively. The pattern was a gradual overall increase to the maximum value in the distilled water experiments occurring at 4 or 8 hours for 500 ml. and 10 or 11 hours for 1000 ml. In the sea water tests, however, diuresis occurred within the first two hours after intubation, reaching a maximum in the third hour in all cases (Figures 5d-5g), and then decreasing to preintubation values. In the sea water tests, the maximum excretion rate varied in direct proportion with the volume ingested and the salt load. Figures 5b and 5c show that with no salt load, the volume intubated is a factor in determining the maximum excretion rate and Figures 5e and 5g indicate the effect of increasing the salt load without changing the volume input.

The concentration of chloride ion in the urine, shown in Figures 6a-6g, varied from 0 to 508 mEq./l. in the experiments. The lowest values were obtained during the control tests and after distilled water intubation, and the highest values, after sea water intubation. In the control, the chloride ion concentration decreased gradually from 13.1 to 5.2 mEq./l. for Seal #1 and from 44.6 to 2.8 mEq./l. for Seal #2. Intubation with 500 ml. resulted in decreases from

FIGURE 5: Changes in urine volume (ml.) with time.
Intubation at 0 time.

- a. Control
- b. 500 ml. distilled water
- c. 1000 ml. distilled water
- d. 500 ml. sea water
- e. 750 ml. sea water
- f. 1000 ml. sea water
- g. 750 ml. $1\frac{1}{2}$ x sea water

Seal #1 ———

Seal #2 -----

Time (hours)

Volume (ml.)

FIGURE 6: Changes in urine chloride concentration
(mEq./l.) with time. Intubation at 0 time.

- a. Control
- b. 500 ml. distilled water
- c. 1000 ml. distilled water
- d. 500 ml. sea water
- e. 750 ml. sea water
- f. 1000 ml. sea water
- g. 750 ml. $1\frac{1}{2}$ x sea water

Seal #1 ———

Seal #2 -----

44.2 to 1.1 mEq./l. in Seal #1, and from 20.3 to 1.0 mEq./l. in Seal #2. Similarly, there were decreases after the intubation of 1000 ml. of distilled water (24.1 to 0.0 and 9.5 to 0.1 mEq./l. in Seals #1 and #2, respectively). The interesting effects were obtained after sea water intubation. The maximum chloride concentration is obtained within the first three to five hours for normal sea water and in the eighth hour for $\frac{1}{2}$ x sea water and is independent of the amount of fluid intubated or salt load (Figures 6d-6g). The shape of the graph varied slightly depending on the amount and concentration of sea water ingested. The chloride ion concentration decreased, but did not return to the preintubation values at any time during the 13 hours.

The changes in sodium ion concentrations (mEq./l.) are shown in Figures 7a-7g. These are very similar to those obtained for chloride ion concentration (Figures 6a-6g). The lowest values occurred in the control tests and following distilled water intake. The range of values over the 13 hours in the control, 500 ml. and 1000 ml. distilled water are as follows: 72.0 to 9.5 (#1) and 34.0 to 13.0 (#2); 49.0 to 8.0 (#1) and 75.0 to 24.5 (#2); 33.0 to 6.0 (#1) and 65.0 to 6.0 (#2), respectively. Following sea water intubation, the maximum concentration of sodium excreted was obtained within the first three to four hours after intubation and this value was approximately the same (470 to 500 mEq./l.) regardless of the amount of concentration ingested. Also, as in the chloride ion analyses, there are

FIGURE 7: Changes in urine sodium ion concentration
(mEq./l.). Intubation at 0 time.

- a. Control
- b. 500 ml. distilled water
- c. 1000 ml. distilled water
- d. 500 ml. sea water
- e. 750 ml. sea water
- f. 1000 ml. sea water
- g. 750 ml. $1\frac{1}{2}$ x sea water

Seal #1 ———

Seal #2 -----

Time (hours)

some differences in the shapes of the curves, but on the basis of the tests done, it is not known whether these are significant. Following $1\frac{1}{2}$ x sea water intubation, both the chloride and sodium concentrations reached their maximum concentrations later and remained closer to their maximum values for the latter part of the test period than the other sea water experiments.

The results of potassium analyses are presented in Figures 8a-8g. It is readily seen that they differ from those patterns obtained in the sodium and chloride analyses. As in the case of urine osmolality, Seal #2 appears to have higher values than Seal #1 in the control. However, it is not possible to know without doing further experiments whether the control values are repeatable. The average value of potassium ion concentration did not appear to change drastically in the distilled water tests when compared to those of the control for Seal #1, whereas, for Seal #2 the control values were very much higher than in the distilled water tests. The ingestion of 500 ml. of sea water resulted in an increase in potassium ion concentration after 4 hours. This could be related to the decreased sodium ion concentration (Figure 7d). In the other sea water experiments, the potassium ion concentration tended either to remain constant or to decrease slightly 4 hours after intubation, except with Seal #1 after 750 ml. sea water ingestion.

In this study, one question of interest is whether the functioning of the kidney and of the body excretion systems

FIGURE 8: Changes in urine potassium ion concentration (mEq./l.) with time. Intubation at 0 time.

- a. Control
- b. 500 ml. distilled water
- c. 1000 ml. distilled water
- d. 500 ml. sea water
- e. 750 ml. sea water
- f. 1000 ml. sea water
- g. 750 ml. $1\frac{1}{2}$ x sea water

Seal #1 —————

Seal #2 -----

are overtaxed by the ingestion of salt water. Does the kidney function efficiently enough to rid the body of the ingested ions without taxing the reserves of body water? Table I presents the total output of fluid, chloride, sodium and potassium as a percentage of the total input. The volume output following distilled water ingestion is from 42-79%. When sea water is intubated, the amount of water required to eliminate all of the sodium and chloride ions is greater than that made available by sea water alone. With $1\frac{1}{2}$ x sea water, more than 100% of the volume ingested is excreted within 13 hours, although not all of the sodium and chloride ions are (94 and 63 - 80%, respectively.) In all cases two to five times the amount of potassium input is excreted within the 13 hours, whereas the percent output of the sodium and chloride ions is between 35 and 69% and 28 and 55%, respectively for sea water experiments. The ingestion of $1\frac{1}{2}$ x sea water is found to result in the excretion within 13 hours of $1\frac{1}{2}$ to 2 times the ion output as compared to the tests using normal sea water.

Table II lists the osmotic and ionic concentrating capacities that have been obtained for the kidneys of the two seals studied. Seal #2 had higher values for osmolality and electrolyte concentrations. The maximum osmolality was attained in Seal #1 two hours after 1000 ml. of sea water were intubated, and in Seal #2, 13 hours after 750 ml. of sea water were intubated, with values of 1700 and 2050 mOsm./l., respectively. The maximum sodium ion concentration occurred in Seal #1 during the fifth hour of the 1000 ml.

TABLE I: Percentage of input of volume, chloride, sodium and potassium excreted in 13 hours.

<u>Seal #1</u>	<u>Water output (%)</u>	<u>Chloride output (%)</u>	<u>Sodium output (%)</u>	<u>Potassium output (%)</u>
500 ml. D.W.	79			
1000 ml. D.W.	55			
500 ml. S.W.	73	50	63	313
750 ml. S.W.	60	49	61	205
1000 ml. S.W.	63	55	69	256
750 ml. $1\frac{1}{2}$ x S.W.	128	80	94	380
<u>Seal #2</u>	<u>Water output (%)</u>	<u>Chloride output (%)</u>	<u>Sodium output (%)</u>	<u>Potassium output (%)</u>
500 ml. D.W.	58			
1000 ml. D.W.	42			
500 ml. S.W.	50	41	44	549
750 ml. S.W.	48	39	49	317
1000 ml. S.W.	35	28	35	193
750 ml. $1\frac{1}{2}$ x S.W.	132	63	94	335

TABLE II: Maximum values recorded for urine osmolality and chloride, sodium and potassium ion concentrations.

O.P.	Max. (mOsm./l.)	U/P	S.W.	U/S.W.
Seal #1	1700	5.07	1099	1.87
Seal #2	2050	5.93	1099	2.25
Na^+	Max. (mEq./l.)	U/P	S.W.	U/S.W.
Seal #1	496	3.08	447	1.11
Seal #2	523	3.02	447	1.17
Cl^-	Max. (mEq./l.)	U/P	S.W.	U/S.W.
Seal #1	472	3.37	448	1.05
Seal #2	508	3.90	448	1.12
K^+	Max. (mEq./l.)	U/P	S.W.	U/S.W.
Seal #1	172	34.4	10	17.2
Seal #2	263	52.6	10	26.3

OPEN SEA WATER (Barnes, 1954)

Cl^- : 548.30

Na^+ : 470.20

K^+ : 9.96

of sea water test and in Seal #2 during the fourth hour of the 750 ml. sea water test, with values of 496 and 523 mEq./l., respectively. The maximum chloride ion concentration was reached in Seal #1 nine hours after intubation of 750 ml. of $1\frac{1}{2}$ x sea water and in Seal #2 four hours after intubation of 500 ml. of sea water. The potassium values were at their maximum 13 hours after 500 ml. of sea water were intubated in Seal #1 and 12 hours after the same in Seal #2.

The urine/plasma (U/P) ratios are presented in Table II. The ratios of osmolality and electrolyte concentrations in the urine to that of sea water are also presented in Table II. These ratios indicate that the chloride and sodium excreted in the urine are present at about the same concentration as in sea water, whereas the potassium ion concentration and osmolality are higher in the urine than in sea water. For comparisons, the ionic concentrations of "open" sea water are listed.

DISCUSSION

Intestinal absorption in man and dog has been shown to be complete within 12 hours after feeding (Davenport, 1961). Although the gastro-intestinal physiology of the seal has not been examined and experiments to determine the rate of passage of food were not attempted, it was assumed that the seal, a carnivore, would be in a post-absorptive state by 36 hours after its last meal. If so, then changes in the plasma and urine after this time would be due primarily to the fluids ingested and absorbed during the experiments rather than by any food remaining in the stomach and intestine.

Gastric evacuation (Hunt, et al., 1951) and absorption in the small intestine (Parsons and Wingate, 1961) have been shown to be slower for hypertonic solutions than for hypo- and isotonic solutions. Therefore, it is probable that distilled water is absorbed more rapidly than the sea water solutions. Retarded absorption of the sea water solutions occurs also because of the presence of magnesium and sulfate ions, which according to Davenport (1961), "... are slowly absorbed, and their presence in intestinal contents prevents water absorption." Changes in plasma osmolality and electrolyte composition reflect the absorption of intubated solutions. In this project, plasma changes after fluid intubation were apparent only after the ingestion of the higher volumes and concentrations of electrolytes possibly because of the large blood volume of marine mammals as compared with terrestrial mammals and the dilution effect.

Smirk (1933) using human subjects, demonstrated that one liter of distilled water was absorbed within 22 to 25 minutes. Klisiecek, et al. (1933) found that absorption of distilled water in dogs took slightly longer. It therefore seems likely that if plasma changes occurred, they should be observed in the seal $1\frac{1}{2}$ hours after intubation. The absence of diarrhea may indicate complete or near-complete absorption. Magnesium and sulfate ions in concentrations found in sea water are emetics for humans (Fletcher, et al., 1943), although they can safely ingest and completely absorb small quantities (Goodman and Gilman, 1965). These seals did not appear to have any trouble absorbing these ions since no diarrhea was observed. Any fecal matter voided was thick, slimy and characteristic of the seal.

In all mammals there are several sites of fluid excretion, such as the eyes, nose, salivary glands and kidneys. The eyes (Thaysen and Thorn, 1954) and salivary glands (Thaysen, et al., 1954) produce fluids with a potassium ion concentration that is hypertonic to the blood plasma. However, it is likely that neither these, nor the nose, played a significant role in excreting excess water or electrolytes. Observations during experiments in which distilled water was intubated indicated no increase of fluid from these areas, whereas in the sea water experiments, fluid output from these areas decreased. Extrarenal sites for water and electrolyte excretion, comparable to those of marine birds and reptiles, have not been reported in mammals. The main

site of osmotic and ionic regulation in the harbor seal can, therefore, be viewed as occurring predominantly in the kidneys.

The volume and composition of the body fluids of mammals are regulated within narrow limits. The regulatory capacities of the kidney can be determined by subjecting experimental animals to such conditions as dehydration, hydration and salt-loading. The osmotic and ionic regulatory capacities of the kidney refer to the ability of the kidney, as dictated by changes in body fluids, to maintain the quantity and quality of substances which are ultimately excreted. When body fluids change in pH, osmotic pressure, ionic composition or volume, mechanisms are initiated by the nervous and endocrine systems which act on the renal system to regain the equilibrium state. Changes in kidney processes, for the most part, involve alterations in the reabsorption and secretion processes, and also in the glomerular filtration rate (GFR). Changes in the GFR are seldom detected in man, but in seals they are frequent and especially marked after feeding (Hiatt and Hiatt, 1942) and during asphyxia (Lowrance, et al., 1956). The overall result of these processes is the production of a urine varying in volume and composition which results in the maintenance of well-regulated body fluids.

Periods of dehydration (the control experiments) resulted in relatively low rates of urine production and electrolyte excretion (sodium and chloride). An average of 14 to 15 ml.

of urine per hour was produced by both seals. These were similar to the quantities determined for periods between meals by Smith (1935), Bradley and Bing (1942) and Schmidt-Nielsen, et al. (1959). Man produces on the average 30 ml. of urine per hour in cases of moderate hydropenia and can drop to 12 to 18 ml. per hour in cases of extreme dehydration (Pitts, 1963). During moderate dehydration the decreased urinary output is due to changes in water reabsorption and not to alterations of the GFR. In more extreme dehydration, the changes in the filtration rate are caused by a reduced blood volume. In the seal, low urine volume during dehydration and starvation has been attributed to increased electrolyte and water reabsorption (Bradley, et al., 1954) and to vasoconstriction with the resultant reduction in the GFR (Hiatt and Hiatt, 1942). It was proposed by the Hiatts that the reduced urine flow between meals enables the seal to conserve its water supply. With low urine output there was also a low electrolyte excretion and a high urinary osmotic pressure. The high osmotic pressure was probably due to a high urea output, which is the major osmotically active substance in the urine during fasting (Smith, 1935). With high antidiuretic hormone (ADH) and aldosterone levels the result is a high permeability of water and reabsorption of electrolytes in the nephrons and a concentrated urine with a high osmotic pressure during dehydration and starvation. Maximum reabsorption is also assisted by reduced GFR which decreases filtration

and filtrate perfusion in all nephrons, resulting in a longer period of contact between filtrate and reabsorbing tubular cells. With the reduction of filtrate, the reabsorptive mechanisms may operate more efficiently in removing solutes. Smith (1936) found that considerable quantities of ammonia, creatinine and creatine were also present in the urine in addition to urea, 24 to 36 hours after the last meal, and contributed to the osmolality.

In the control tests, urine volumes were between 14 and 15 ml. per hour and the concentrations of sodium and chloride ions were low, indicating a relatively constant output of osmotically active particles requiring a certain quantity of water, and also a near-maximum reabsorption of filtered water and these two electrolytes. The differences between the two seals in Figure 2a may be accounted for by different kidney sizes, levels of organic substance excretion, urine concentrating abilities or different degrees of dehydration before the tests. The concentrating abilities of the two seals are shown in Figure 2a, in which Seal #1 concentrated its urine to a maximum of 1550 mOsm./l., whereas, Seal #2 concentrated its urine as high as 2050 mOsm./l. Urine potassium ion concentrations in the control were relatively higher than other electrolytes and were probably due to the movement of potassium out of cells as a result of water movement from intracellular to extracellular fluids, required to maintain a constant volume of extracellular fluid during periods of dehydration. The effects of stress

on potassium secretion (Pitts, 1963) and possible tissue damage may, in part, account for the relatively high concentrations of this ion.

When fluids were intubated, there was a diuresis within two hours, a water diuresis with distilled water and an osmotic diuresis with sea water. The pattern of urine volume changes after distilled water intubation was similar to the excretory pattern in man and dog after ingestion of an isotonic solution (Pitts, 1963), in that the increase in urine volume was gradual. An abrupt change normally occurs in man and dog after ingestion of hypotonic solutions. Albrecht (1950) and Bradley, et al. (1954) report a diuretic pattern for the seal similar to those of the dog and man. No measurements were made to determine whether the GFR changed during any of the tests. However, Bradley, et al. (1954), Page, et al. (1954) and Schmidt-Nielsen, et al. (1959) found that the GFR did not change during electrolyte and water loading. On the basis of their results, it seems probable that if changes in filtration rate did not occur, then the changes in electrolyte concentrations and in urine volume were due to alterations in the reabsorptive and secretory processes in the kidney. The increase in urine volume could be attributed to a decreased level of antidiuretic hormone which would increase water output. The seals may have been dehydrated, even though they were in fresh water up to the time of the experiment and had the opportunity to increase their body water, since they retained from 21 to

50% of the intubated water over 13 hours. The reduced GFR that occurs in the seal during starvation may also have contributed to the low water output.

The intubation of hypertonic solutions resulted in an increased urine volume up to 210 ml. per hour within three hours. The patterns of volume change for sea water and hypertonic salt solutions were similar to those reported by Albrecht (1950) and Bradley, et al. (1954), respectively. The total electrolyte excretion in the sea water experiments increased with the electrolyte load. The increased volume of urine and electrolyte concentrations could be accounted for by decreased aldosterone levels during the early stages which would result in decreased sodium reabsorption and an osmotic diuresis. In the latter stages of the experiments the decreased electrolyte and urine volume can be explained by increased aldosterone levels which would increase electrolyte and water reabsorption. Similar effects due to varying levels of aldosterone have been demonstrated in the dog (Roemmelt, et al., 1949).

In mammals, as the urine volume increases, the osmotic pressure decreases if there is no increase in excretion of osmotically active particles. With a decreased urine volume, there is an increased proportion of solutes in the urine and thus the osmolality increases. The changes in osmotic pressure during the experiments can thus, in part, be explained in terms of urine volume and the proportion of osmotically active particles present. Figure 2 presents

a comparison of the specific gravity and osmotic pressure values obtained in the distilled water experiments. It can be seen that there is a great similarity in the changes occurring in these values. The changes in sodium, chloride and potassium ion concentration discussed previously are not great enough to account for the large change in osmolality after intubation of distilled water. It is therefore probable that the amount of total solids (urea being the major substance, Koch, 1965) in the urine is the predominant factor determining the osmotic pressure. Schmidt-Nielsen, et al. (1959) found that the concentration of urea decreased after infusion with distilled water and the urine volume increased. A corresponding change between specific gravity and urine volume occurred in these experiments also.

The osmotic pressure of the urine decreased in the distilled water experiments, but at no time was it hypoosmotic to the plasma. The failure of the urine to become hypoosmotic in the seal may be accounted for by assuming that even though there was a decreased electrolyte excretion, the other major constituents of the urine (urea, ammonia, creatine and creatinine) were present in sufficient concentration to maintain the recorded osmotic pressure. Also influencing the osmolality was the gradual increase in the urine volume, within which there was a larger proportion of osmotically active particles than there would

have been if an abrupt increase had occurred. If the pattern of water diuresis was similar to that observed in the dog (Pitts, 1963) then the proportion of particles in the urine perhaps would have been low enough to make the urine hypoosmotic to the plasma.

In the sea water experiments, the urine osmolality values remained relatively constant for Seal #1, but increased for Seal #2 over the 13 hours following intubation (Figures 2d-2g). Figures 6d-6g and 7d-7g showed that the chloride and sodium concentrations increased rapidly after 1 to 2 hours and then remained constant or decreased. Therefore, there does not appear to be an obvious major contribution to osmolality by these ions since a large increase in ion concentration should have been reflected by a similar increase in osmotic pressure. However, if the specific gravities of the urine samples are plotted (Figure 2d-2g) and related to ion concentrations and urine volumes, the osmotic pressure trends can be explained. With an increase in urine volume, the concentration of solids decreases, as reflected by the decrease in specific gravity. This statement is based on the fact that the time of the greatest decrease in specific gravity occurs at the same time as diuresis, and also that Seal #1 with a greater urine volume, has the lower specific gravity. However, at this time, the ion concentrations greatly increase, thus counteracting the decrease in osmotic pressure expected by a decrease in

the concentration of solids excreted. The net result is an osmotic pressure that does not drop, but increases with time. This latter increase is probably due to the fact that the ion concentrations remain maximal or decrease slightly while the specific gravity increases. The increases in specific gravity could be due to an increased water reabsorption in the latter part of the experiments, as reflected in the decreased urine volume. The specific gravity values also provide a possible explanation for the differences between the osmotic pressure values of the two seals at the end of the experiment, since the specific gravity values are different in the same direction as the osmotic pressure values, whereas the ion concentrations are very similar.

The concentrating capacity of the kidney indicates how the seal is able to meet its water requirements, whether, in fact, it can drink sea water and remove the electrolytes without body dehydration or whether it gains all of its water from its food. The seal can concentrate sodium and chloride to approximately the same concentrations found in open sea water (Table II), this indicates that the seal can gain little or no water by drinking sea water. In fact, this is the case, as can be seen in Table I. In order to eliminate all of the sodium and chloride introduced with sea water the seal will have to draw on body water.

Further evidence to indicate that this seal cannot benefit from drinking large volumes of sea water is found

in the inability of the animal to eliminate both the water and electrolytes in sea water. Bradley, et al. (1954) state that in the periods between meals, filtration as well as water and electrolyte excretion decreases, in fact, "... tubular activity may fail to operate in accordance with body needs, i.e., water and salt retention occurs despite the need to eliminate loads imposed experimentally." The results of the present experiments support this suggestion in that the seal retained from 27 to 65% of the water, from 45 to 72% of the chloride and from 31 to 65% of the sodium after thirteen hours. The results suggest that by continued drinking of sea water, the animal may become edemic and increase its body electrolyte concentration to toxic levels. If the animal takes in small quantities of sea water, as it may do when feeding, there would be sufficient isorrheic water provided, and with post-feeding hyperfiltration the body would be able to excrete the excess electrolytes from the sea water. However, if the animal ingests sea water and does not feed, the vasoconstriction and reduced GFR would cause the water and electrolytes to be retained in the body. Since this animal spends one-third of its time submerged (Schmidt-Nielsen, et al., 1959), during which the GFR, water and electrolyte output, and respiratory water loss are reduced, and also during which there is little water loss by evaporation and in the feces, then the possibility of body hydration seems just as great a hazard as electrolyte intoxication following frequent ingestion of large quantities of sea water.

SUMMARY

1. The osmotic and ionic regulatory capacities of the kidney have been determined for two female harbor seals during periods of dehydration and water and salt loading.
2. The maximum values obtained for urinary osmotic pressure, and sodium and chloride and potassium ion concentrations are 2050 mOsm./l., 523 mEq.Na⁺/l., 508 mEq.Cl⁻/l. and 263 mEq.K⁺/l., respectively.
3. Distilled water ingestion results in an increase in urine volume and decrease in osmotic pressure and ion concentrations.
4. Following sea water ingestion, a large increase in urine volume occurs within two hours, urine osmolality remains constant or increases, the sodium and chloride ion concentrations increase to their maximum values within 3 to 5 hours, and the potassium ion concentration behaves differently from these ions.
5. The concentrations of sodium and chloride in the urine are approximately equal to those of full strength sea water, indicating that no water can be gained from its ingestion. In order for the seal to eliminate these electrolytes it must draw on its body water.

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