THE EFFECTS OF HYPERTONIC SODIUM CHLORIDE INJECTION ON BODY WATER DISTRIBUTION IN DUCKS (*Anas platyrhynchus*), GULLS (*Larus glaucescens*), AND ROOSTERS (*Gallus domesticus*)

by

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ABSTRACT

Isotope and dye estimates were made of body fluid compartment sizes in White Leghorn roosters, Glaucous-winged gulls, and in groups of Pekin ducks which were raised on either fresh water or regimes of hypertonic NaCl solution. The gulls and both groups of ducks were observed to have plasma (T-1824 dye) and total body water (H_2^3O) volumes larger than those of the roosters, whereas the reverse was true for Br^{82} space (extracellular fluid; ECF) measurements. Salt fed ducks showed smaller, but insignificantly different compartment sizes (% body weight) when compared to fresh water raised ducks.

The effects of an intravenous injection of hypertonic NaCl on the distribution of body water were compared among birds which differed in their capacity for renal and extra-renal salt elimination. In those birds (gulls, salt water ducks, and fresh water ducks with functional salt glands) which exhibited extra-renal salt secretion, the increase in ECF was significantly greater in response to the intravenous injection of hypertonic NaCl than in those birds (roosters and non-secreting fresh water ducks) which did not utilize the salt glands.

The relative amounts and concentrations of the salt load removed by renal and extra-renal routes of elimination were compared. Birds with actively secreting nasal glands voided a major equivalent of the injected NaCl as solutions hypertonic to plasma NaCl levels. Renally eliminated NaCl represented a much smaller portion of the load and was in all cases hypo- or isotonic with plasma ion levels. Isotopically labelled $Na^{22}Cl$ administered concomitantly with the salt load in several of the test birds revealed that a large portion of the labelled sodium chloride was removed by the nasal glands and kidneys before there was equilibration of the injected load with extravascular compartments.

A preliminary report is made on the composition and possible source of an excess eye secretion observed in the rearing of saline fed Pekin ducks. The enlarged Harderian glands of these birds were implicated as the source of a fluid several fold hyperkalemic to plasma ion concentrations. The secreted fluid was observed to accumulate and encrust the feathers below the inner canthus of the eye.

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INTRODUCTION

Maintenance of osmotic and ionic balance in response to variations in the body fluids of higher vertebrates, such as the bird, are primarily the function of the kidney. The kidney may, however, be significantly aided in some forms by various extra-renal excretory routes or by behavioral adaptations. The contributions of these mechanisms to total osmoregulation in birds have been described in considerable detail by the work of Knorr (kidney), Schmidt-Nielsen (salt gland), Farner and Bartholomew (respiratory evaporation, diet tolerances, and regulatory behavior), and others. In contrast to the abundance of information describing the mechanisms and limits of these physiological processes, the underlying stimuli for regulatory responses accompanying tonic or osmotic changes are less well characterized. Since tolerance and capacity for change are the central factors in adaptation and homeostatic control, limitations in the size and ionic composition of body fluid spaces must be considered as the most fundamental indexes for both the need and extent of osmoregulation.

A surfeit of recent information in this regard has directly implicated changes in extracellular fluid (ECF) volumes with the regulation of hormonal output. For example, increases in ECF volume in dogs stimulate the release of aldosterone and antidiuretic hormone (Bartter, 1963). A hormonal effect involving ECF volumes has been observed in roosters in which the injection of estrogens affected an increase in ECF volume which was thyroxin reversible

(Gilbert, 1963). Other studies which have demonstrated basic diurnal changes in ECF volume of humans and seasonal ECF volume changes in Arctic huskies (Hannon and Durrer, 1963) further indicate that the size of this compartment is a labile parameter and as such may well represent an important link between osmotic conditions of the cellular milieu and neural or hormonal tranducers of regulation.

Extracellular fluid space changes in birds have also been associated with the initiation of nasal gland activity in ducks and seagulls. Holmes (1965) has reported that salt-loading in ducks resulted in secretion of nasal fluid in conjunction with marked increases in plasma volume. McFarland (1964) has measured the minimum NaCl load required to elicit nasal gland secretion in a variety of seagulls and has found that the injection of 0.49 gm NaCl per kilogram of body weight resulting in an average plasma ion elevation of 21 mEq/l Na^+ and 30 mEq/l Cl^- stimulates gland secretion. Hormonal studies by Holmes, Phillips and Butler (1961) in which injection of adrenal corticoids (aldosterone and cortisol) initiated nasal gland activity prior to a rise in plasma Na^+ level have been used to suggest that nasal gland stimulation may be affected by these agents. The authors suggest that the hormones are normally released prior to secretion in response to elevated plasma Na^+ concentration. Evidence from dogs and humans, however, has shown that regulation of aldosterone secretion occurs independently of extracellular ion or water concentrations, but is determined instead by changes in ECF volume (Bartter et al. 1956; Bartter et al. 1959; Bartter, 1963).

The increasing implications of ECF volume changes as possible stimuli for osmoregulatory reactions point to the potential usefulness of extracellular compartment parameters as portents of a variety of physiological responses. The following study was undertaken for the purpose of characterizing intracellular and extracellular body compartment sizes. For the purposes of this work extracellular fluid (ECF) volume is comprised of the circulating plasma and lymph, the interstitial fluid, and the sequestered fluids with which these freely exchange. Intracellular fluid is that volume within cells, which together with the extracellular fluid, comprise the total body water (TBW). Three avian species were examined for accomodation and change in each compartment following intravenous injection of a hypertonic NaCl solution. A correlation between ECF compartment change and osmoregulatory function of the kidney and nasal gland was sought in birds which vary in their ability (roosters versus gulls), need (fresh water versus salt water fed ducks), and normal potential (salt adapted ducks versus gulls) for regulation by these two mechanisms. A preliminary report is also given on observations of an excess eye secretion in salt fed ducks.

MATERIALS AND METHODS

Source and Maintenance of Birds

White Pekin ducks, domesticated form of Anas platyrhynchos, were obtained from a commercial source two days after hatching and were subsequently maintained for five days on "duck grower mash" and an ad libitum supply of fresh tap water. At the end of this period the ducks were randomly grouped; one half (30 birds) continued on a fresh water regime and the other half had NaCl added to their drinking water at a rate of approximately 4.0 gm per liter per week to a maximum concentration of 0.483 M NaCl (0.557 M NaCl = osmotic equivalent of full strength sea water; Prosser and Brown, 1961). These ducks, designated salt ducks, were maintained at this concentration for at least 45 days prior to their use, during which time they had continuous access to feed and saline solutions. All ducks were 90 - 100 days of age and of mature plummage at the time of experimentation. Both groups of ducks were maintained indoors at normal room temperature in mesh bottom animal pens housing six ducks each.

The Glaucous-winged gulls, Larus glaucescens, were caught locally using smelt injected with approximately 100 mg of sodium pentobarbital (Abbott Veterinary Nembutal). Their body weights were comparable to those of six month old birds reported in other studies on Larus glaucescens of the same locale (Holmes et al. 1961). They were fed a ground fish and cereal base commercial cat food (Puss'n Boots) and given an *ad libitum* supply of fresh tap water during the two weeks prior to their use. Housing was provided in an outdoor cage under natural temperature and light conditions. Plasma ion levels and total body weights at the time of the experiments were only slightly below those recorded on the day of their capture.

Mature White Leghorn roosters, *Gallus domesticus*, were obtained from the Department of Poultry Science and were maintained for at least one week in indoor pens with commercial chicken feed and fresh tap water *ad libitum.*

All birds were repeatedly handled during weighings and blood samplings throughout their rearing in a manner that approximated the technique used in experimental conditions. Birds were fasted, but permitted access to their water sources during the twelve hours preceding experimentation.

Sampling Procedures - Plasma, Cloacal Fluid, Nasal Gland Secretion and Eye Fluid

Prior to the start of each run a femoral vein was catheterized under local anesthetic (topical application of 2% Procaine HCl + 1:20,000 Epinephrine-Winthrop Laboratories) and P-90 polyethylene tubing inserted to a point in the upper leg. This catheter served as the sampling and injection route throughout the test period. The degree of contamination resulting from introducing and sampling via the same catheter was determined by comparison with a bird in which injection and sampling were carried out on opposite legs.

Dye, ion, and isotope dilution values were comparable to single catheter preparations. To insure against residual contamination, however, all syringes used to inject concentrated standards were flushed with 1.0 - 2.0 ml of circulating blood. A heparinized isotonic saline solution was used to return blood to the end of the catheter, thereby reducing dead space between samplings. A single catheter was used as the preferred sampling technique in order to minimize operational trauma and to maintain a more normal blood flow to the extremities. At sampling time the heparinized saline was withdrawn and a volume of blood (0.2 ml) permitted to drain from the catheter before a sample (0.5 ml) was collected. Blood samples were taken from each of the birds at regular intervals 2, 4, 8, 16, 30, 60 and 120 minutes after NaCl loading (0.2% body weight of 5N NaCl solution - v/w), and at intermittent sampling periods concomitant with cloacal discharges and nasal fluid collection intervals. The total blood loss from cannulation and sampling during the course of each experiment is estimated to have been from 6 to 8 ml. Duplicate hematocrits were taken of each blood sample using Strumia microhematocrit tubes (32 x 0.8 mm) which were spun at 3000 rpm in a Model HN International Equipment Company (IEC) centrifuge for 30 minutes; plasma was obtained from whole blood samples by centrifugation at 6000 rpm for 10 minutes.

During the experimental procedure the birds were placed in canvas slings and suspended in fruit crates modified to maintain them in an erect and nearly normal standing posture. Their wings were immobilized on foamcovered boards and their legs, which protruded from the sling, were secured to minimize movement. Cloacal samples were collected by positioning a large plastic funnel under their tails and catching spontaneously voided samples in weighed glass vials. The volume (estimated from weight) and time of collection were recorded for each sample. Nasal gland secretion samples from ducks and gulls were collected with a minimum of evaporation by inserting the upper bill and nares into weighed glass collecting vials which were afterwards sealed with airtight tops and stored under refrigeration for later analysis. Post-salt load collections lasting 15-20 minutes each were continued for one to two hours after injection, or until dripping had ceased. Samples of eye fluid (tears) were taken with a micropipette from the inner canthus of the eye immediately after a drop of fluid appeared.

Ion and Isotope Analysis

The sodium and potassium concentrations of urine, drip, tear, and plasma samples, which had been diluted to analyzable concentrations with glass distilled water and refrigerated for periods of 24 to 72 hours, were determined with the use of a Zeiss PF 5 flame photometer. Chloride determinations were made using a Buchler-Cotlove chloride titrator. The radioisotopes used in this experiment [Na^{22} (2.8 mC/mg Na^+); Br^{82} (1.23-2.20 mC/mg Br^+); and $\mathrm{H}_2^3\mathrm{O}$, (1.0 C/mg H^+)] were obtained from Cambridge Nuclear Corp., Boston, Mass. Doubly and triply labelled plasma samples (0.1 ml) were dissolved in Bray's solution (15 ml) and counted on a Mark I

Nuclear of Chicago liquid scintillation spectrometer. High energy Br^{82} ($B^- = 0.44$ Mev.) and Na^{22} ($B^+ = 0.5$ Mev.) disintegrations were counted simultaneously with the same window settings of Channel C while lower energy tritium disintegrations were measured in the lower spectrum settings on Channel A. Na^{22} and Br^{82} counts were further separated in triply labelled samples by recounting after 10 days when the disintegrations of remaining Br^{82} were reduced to background levels. [Half life (t 1/2) Br^{82} = 35.7 hours; t 1/2 Na^{22} = 2.6 yrs.; t 1/2 H^3 = 12.5 yrs.] The significance of the overlap of lower energy Na^{22} and Br^{82} disintegrations into the tritium spectrum was reduced by maintaining H_2^3O at an approximately ten fold greater concentration. ($Na^{22} + Br^{82}$ = 1500 cpm; H_2^3O = 10,000 cpm). Samples of drip, cloacal fluid, and standard solutions (0.01 ml) were quenched to the same degree as blood samples by the addition of 0.1 ml of unlabelled plasma. Two consecutive one minute counts were made on each sample and the average of these recorded as the final counts per minute (SD = ± 1 to 2%).

Body Compartment Estimates

The dilution technique for measuring body compartment sizes is based on the assumptions: 1) that the tracer substance mixes rapidly and uniformly with the fluid and the compartment to be measured, 2) that its presence in no way affects the physiological state of the compartment or the animal, and 3) that it remains exclusively in the domain of the space being measured.

a) Plasma Volume Determinations. One milliliter of a 1.0% T-1824 (Evans Blue) dye solution was injected with a calibrated syringe one hour prior to salt loading and blood samples were taken subsequently at 5, 10, and 60 minutes after dye injection. The concentration of the circulating dye was determined from standards made by diluting aliquots of the concentrate (1.0% T-1824) with volumes of the animal's plasma obtained 36 hours prior to the experiment. A constant volume of plasma (0.1 ml) together with varying amounts of dye concentrate were diluted to 2.0 ml with physiological saline (155.0 mEq/l NaCl and 5.0 mEq/l KCl) and percent transmittance read on a Bausch and Lomb Spectronic 20 colorimeter at 620 mu. Estimates of the dye concentration, when mixing had been completed, were made by extrapolating the disappearance rates of the dye to time zero on semilogarithmic plots of concentration (µg/ml) versus time (minutes), according to the method of Dawson et al. (1920). The volume of circulating plasma at time zero was then calculated using the known concentrations of dye before and after mixing.

$$\text{Plasma volume (ml)} = \frac{\mu\text{g of T-1824 injected}}{\mu\text{g T-1824/ml plasma at time zero}}$$

Certain corrections are therefore necessary in evaluating the actual behavior of T-1824. The rapid and stoichiometric binding of T-1824 to molecules of serum albumin makes it a particularly suitable indicator of plasma compartment size, but because of its observed accumulation in

Küpfer cells and other macrophage centers and the known leakage of plasma proteins into the lymphatic system, the disappearance rate of T-1824 is significant and measurable (Moore et al. 1943). Since the disappearance rate of T-1824 under relatively unaltered physiological conditions has been shown to be fairly constant, corrections for its loss during the initial distribution period before mixing is complete may be made by extrapolating the slope of the linear portion of the loss rate to time zero (Dawson et al. 1920). Estimates of plasma volume using T-1824 have been shown to compare favorably with values obtained using other methods (I^{131} - Serum Albumin = RISA; Huggins et al. 1963). Plasma samples which gave evidence of hemolysis were excluded from colorimetric determinations.

b) Extracellular Fluid and Total Body Water Determination. At the start of each experiment 1.0 - 1.5 ml of a mixture of $NaBr^{82}$ (35.0 μc) and H_2^3O (5.0 mc) was sampled in duplicate (0.01 ml) and the remainder injected with a calibrated syringe via the indwelling catheter and allowed to equilibrate with the bird's chloride and exchangeable hydrogen spaces. Based on observations of equilibration times in pilot experiments and in control birds comparable in weight to experimental birds, mixing of Br^{82} was complete after 1.0 to 1.5 hours and mixing of H_2^3O after 1.5 to 2.0 hours. Estimates of pre-salt load ECF (extracellular fluid) and TBW (total body water) volumes were made on plasma samples taken at various intervals after the initial mixing period. The approximation of these volumes based on the distributions of tracer isotopes in their respective spaces followed the general formulae:

ECF:

$$\text{Br}^{82}\text{ space (ml)} = \frac{(\text{total cpm Br}^{82}\text{ injected}) - \begin{array}{r}(\text{total cpm Br}^{82}\text{ excreted}\\ \text{in cloacal fluid and drip})\end{array}}{\text{cpm Br}^{82}\text{ per ml plasma sample}}$$

TBW:

$$\text{H}_2^3\text{O space (ml)} = \frac{(\text{total cpm H}_2^3\text{0 injected}) - \begin{array}{r}(\text{total cpm H}_2^3\text{0 excreted}\\ \text{in cloacal fluid and drip})\end{array}}{\text{cpm H}_2^3\text{0 per ml plasma sample}}$$

Blood samples were often taken in the periods between normally infrequent and sporadic cloacal fluid collections. To correct for tracer loss in urine being formed and stored during these intervals it was assumed that the rate of loss, due principally to urine formation (there was no evidence of fecal contamination in most cases), was constant in these intervening periods. The total counts per minute (cpm Br^{82} or H_2^3O) lost in urine formed prior to a blood sampling at time "t", t minutes after the injection of isotopes, was then calculated in the following manner:

$$\begin{array}{l}\text{Total cpm (Br}^{82}\text{ or H}_2^3\text{O)}\\ \text{excreted in urine formed}\\ \text{prior to time "t" after in-}\\ \text{jection of isotopes}\end{array} = \left[\frac{(\ C_2\ )}{\begin{array}{c}(t_2 - t_1)\\ \text{min.}\end{array}} \quad \text{X} \quad \begin{array}{c}(t - t_1)\\ \text{min.}\end{array}\right] + C_1 + C_0$$

t = time of blood sampling between cloacal fluid excretions C_1 and C_2.

C_1= total cpm (Br^{82} or H_2^3O) in cloacal fluid discharge preceding blood sample.

C_2= total cpm (Br^{82} or H_2^3O) in first cloacal fluid discharge given after blood sample.

t_1 = time at which C_1 was collected.

t_2 = time at which C_2 was collected.

C_0 = total cpm (Br^{82} or H_2^3O) excreted in cloacal fluid prior to discharge at time t_1.

The same procedure was used in estimating the total cpm (Br^{82} or H_2^3O) lost through nasal gland secretion when a given blood sampling occurred within a drip collection interval. The total cpm (Br^{82} and H_2^3O) lost at the time of any blood sampling represented the sum of drip and cloacal fluid losses. Average pre-salt load values for estimates of ECF and TBW in each bird were obtained from multiple plasma samples taken over a period of 3 - 5 hours prior to salt injection. Repeated sampling (2 - 5) during this period between isotope injection and salt loading indicated that the size of these fluid spaces remained relatively constant under nonstress conditions. The constancy of these spaces was additionally confirmed for periods lasting the duration of an average experiment (6 - 7 hours) in two salt water and two fresh water ducks.

Because of the short duration of these experiments, during which time the predominant movement of Br^{82} was assumed to be among extracellular spaces, there were no corrections made in any of the Br^{82} space volumes for intracellular equilibrations. Inherent in all group comparisons was the assumption that the presence of this error was standard throughout treatment and species differences. The changes in Br^{82} space volumes have been expressed, for purposes of comparison, as the difference between the average pre-salt load volumes and that of the maximum volume observed in a sixty minute period after salt injection. Estimates of the intracellular fluid volume (ICF) during pre- and post-load periods were obtained by subtracting Br^{82} space from H_2^3O space volumes.

In several of the experiments radioisotopic $Na^{22}Cl$ (5-15 mC) was mixed with unlabelled NaCl in preparing the hypertonic load medium. The presence of Na^{22} label in cloacal fluid and nasal drip samples of birds responding to the load provided a comparison of the percentage of Na^{22} excreted by these two routes.

Gland Weight Determinations

At the completion of the experimental procedure birds were sacrificed by decapitation. Harderian glands, nasal glands, and kidneys were removed, trimmed of connective tissue, and weighed to the nearest milligram on a Mettler torsion balance. Dry weights were obtained by heating tissues to constant weight in a 150 °C oven for 24 hours and reweighing. Wet and dry weights were compared in fresh and salt water fed ducks and are reported in corrected form as gm or mg% body weight.

RESULTS

Measurements of T-1824, Br^{82} and H_2^3O Spaces

a) Plasma Volume. Estimates of plasma volume by the Evans blue (T-1824) dye method have resulted in reproduceable approximations of this compartment size which were consistent with previously published determinations for two of the groups tested. Tables I and II indicate the results of measurements for two White Leghorn roosters ($\bar{x}$ = 4.4 gm % body weight). Estimates of plasma volume in fresh water reared Pekin ducks for the control period prior to salt loading yielded somewhat larger volumes ($\bar{x}$ = 6.0 + 0.3%).

Plasma volume relative to body weight appears to be significantly ($p = < 0.05$) increased in the group of ducks receiving salt supplemented water (6.5 + 0.9%) as opposed to fresh water ducks (5.7 + 0.5%). Because of the large differences in body weights between saline and fresh water fed ducks (Table I) it appeared more desirable to compare plasma volumes with reference to a standard that was less treatment variant than body weight. Estimates of total body water from H_2^3O space measurements were less subject to saline effects, as evidenced by the statistically insignificant ($p = < 0.10$) correlation of H_2^3O space volumes with body weights in the two groups of ducks (Table I). A comparison of plasma volume as a percentage of the H_2^3O space did not substantiate the difference with respect to body weight seen between fresh and salt water ducks (Table II). The use of H_2^3O space is considered here the more meaningful index for comparison both because of its reduced bias and its general relevance to all phases of body fluid distributions.

In the gulls used in this experiment, estimates of plasma volume based on body weight and H_2^3O space were $\bar{x} = 7.16\%$ and $\bar{x} = 8.14\%$ respectively. These values, like those of the ducks, were considerably larger than the volumes found in roosters (see Table II).

b) Extracellular Fluid. Br^{82} space measurements as approximations of extracellular fluid volume likewise, were made during the periods prior and subsequent to salt loading in each of the test groups. Estimates of the size of this compartment in the various groups were made simultaneously with other isotopic measurements of compartments during the post equilibration period prior to salt loading (1 - 2 hours after injection). Table I indicates the relative proportions of Br^{82} space means and Table II the Br^{82} space: body weight and Br^{82} space: H_2^3O space ratios. A comparison of Br^{82} space volumes relative to body weight (Table II) indicates a somewhat larger volume for this compartment in gulls. As indexed against individual H_2^3O spaces, however, group differences assume another pattern with the ratios of salt water ducks (41.3%) and gulls (43.6%) being intermediate between those of fresh water ducks (36.4%) and roosters (52.9%). The relevance of these two patterns will be discussed later with reference to possible physiological implications in salt loading effects.

c) Total Body Water. Estimates of total body water in roosters ($\bar{x}$ = 54.3 gm % body weight) were considerably smaller than H_2^3O spaces observed in the gulls (86.8 and 89.1%) and slightly smaller than those of either the fresh water (68.5%) or salt water ducks (64.0%).

Effects of Hypertonic NaCl Injection on Br^{82} and H_2^3O Space Volumes

The nature of equilibrated isotope space responses to intravenous injection of hypertonic NaCl are given in Table III and illustrated in figures 1-5. When the magnitudes of Br^{82} space changes are compared (Table III) a significant difference ($p = < 0.01$) is observed between the mean increase of fresh (10.59%) and salt water ducks (25.49%) which parallels the difference observed in changes of the roosters (16.82%) and gulls (33.09%). The difference between fresh and salt water ducks is broadened and a suggestion as to its physiological significance made when the nasal fluid secreting fresh water ducks (C - 6#3 and C - 3#3) are grouped with the salt fed ducks. The average percent change in Br^{82} space of all ducks responding to the salt load by secretion of nasal gland fluid (23.6% ± SE. = 2.11%) was substantially higher than those of non-secreting ducks (5.1% ± SE. = 1.55%). A plot of the percent change in Br^{82} space against the volume of collected nasal drip (Figure 6) suggests the possibility that a critical volume change may be associated with initiation of nasal gland activity. There is no correlation between the magnitude of change and the volume of secretion as shown in this figure.

Figures 1 - 5 depict the time course of changes in Br^{82} space, H_2^3O space, intracellular volume, and plasma Na^+ concentration for pre- and post-salt load intervals in secreting (Figures 2, 3, 4) and non-secreting (Figures 1, 5) fresh and salt water ducks, seagulls, and roosters. Although plasma Na^+ levels markedly increased in all of the birds as a result of the hypertonic NaCl injection, Br^{82} space expansions vary from increases of 2.1 - 7.3% in fresh water ducks (without nasal secretion) to increases of 18.5 - 38.1% over pre-salt load volumes in gulls and ducks with visible nasal gland secretion. All of the birds in this last group (Figures 2, 3, and 4) were observed to have Br^{82} space increases that occurred maximally within 10 - 15 minutes after injection, thereafter declining to near pre-salt load volumes. All of the observed increases represented substantial changes over mean pre-salt load volumes.

Hematocrits of blood samples taken throughout the pre- and post-salt load periods were in general agreement with the patterns of Br^{82} space changes, although the extent of these changes did not appear to mimic the individual variations in percent change of this space.

Sodium Elimination

Collection of cloacal excretion and nasal drip for period of 2 - 3 hours subsequent to salt loading enabled a comparison of the relative efficiency of sodium elimination by the various birds.

a) Nasal Gland Secretion. The percentage of injected sodium voided via nasal secretion can be seen (Figure 7 and Table IV) to be highest among the gulls ($\bar{x}$ = 61.7%) and substantially higher in salt water ducks ($\bar{x}$ = 31.1%) than in fresh water ducks with active glands ($\bar{x}$ = 1.5%). The observed Na^+ concentrations in the secretions of these three groups are consistent with the most active glands producing the highest concentrations. (Maximum Na^+ concentrations: gull = 1020 mEq/l; salt water duck = 844.5 mEq/l; fresh water duck = 587.9 mEq/l). The percentage of Na^+ eliminated by the nasal gland in secreting fresh water ducks suggests, however, that the principle difference is due to the absence or discontinued presence of secretory stimuli in these fresh water birds. The collection of drip with Na^+ concentrations comparable to those from salt water ducks (600 mEq/l), and the observation of drip in response to a second salt load at the end of the experiment in those birds not responding to the initial load, confirmed the presence of active or potentially active salt glands in all of the fresh water ducks.

A comparison of the percentage of injected Na^{22} eliminated with the total nasal gland sodium secretion in actively secreting ducks and gulls (Table IV) shows that 14 - 16% of the labled sodium injected appears in the sodium secretion. Barring the possibility of isotope discrimination, these findings indicate (as illustrated by salt water duck C - 1#1, Table IV) that the high specific activity of Na^+ found in the nasal secretion (1.6×10^5 cpm/mEq Na^+) relative to that of the injected load (5.8×10^5 cpm/mEq Na^+) or relative to the potentially equilibrated Br^{82} space volume (1.08×10^5 cpm/mEq Na^+) is the result of a rapidly responding concentration mechanism which effectively acts to remove a significant portion of the immediate source of osmotic stress. (Na^+ content of Br^{82} space estimated from pre-salt load Na^+ concentration of plasma and measured volume of Br^{82} compartment.)

b) Cloacal Excretion. Elimination of sodium via renal mechanisms, as measured in cloacal discharge samples (Table IV), was the greatest for roosters which lack any extra-renal route of elimination. The average results from two birds show that 19% of the injected Na^+ load was eliminated cloacally. The gulls in comparison, although less dependent on renal excretion, showed a relatively high percentage of cloacal Na^+ loss (13%) in contract to both groups of ducks (3%). The maximal concentration of NaCl in the cloacal discharge of all the salt loaded birds was at most only slightly hypertonic to plasma. Table V gives sodium and chloride concentrations for representative examples from each group for concurrently sampled post-salt load plasma, nasal drip, and cloacal fluid.

The effectiveness of renal mechanisms in ridding ionic excesses, as seen in the roosters (R-2, Table IV), compares favorably with the nasal gland in the percent of injected Na^{22} voided from the body (14.7%). The specific activity of labelled sodium in this fluid (0.59×10^5 cpm/mEq Na^+), as compared to that of the original salt load (1.77×10^5 cpm/mEq Na^+) and that of the potentially equilibrated Br^{82} space volume (0.41×10^5 cpm/mEq Na^+), also indicates an effective removal probably via a diuretic-like response. The larger volumes and lower urine concentrations (93.3 - 112.8 mEql/ Na^+) underscore the relative inefficiency of this mechanism for conserving body water. The renal response of the non-secreting fresh water duck (C - 6#5; Table IV) is seen to be comparable to that of the rooster in the Na^+ concentration of the collected cloacal fluid (77.1 - 107.0 mEq/l). Comparable too were the specific activity proportions of their renally excreted Na^{22} (1.20 x 10^5 cpm/m Na^+) relative to the Na^{22} injected (5.04 x 10^5 cpm/mEq Na^+) and the potentially equilibrated extracellular Na^{22} (1.04 x 10^5 cpm/mEq Na^+). The concurrence of higher specific activities of Na^{22} in extruded fluids from both routes of elimination as compared to potentially equilibrated extracellular sodium, indicates that both mechanisms are actively removing sodium before the injected load has equilibrated with the Br^{82} space.

Eye Drip and Gland Weights

The observation of moist feather patches radiating from the anterior corner of the eye into the region below the nasal canthus in salt water fed ducks (Figure 8) promoted a preliminary examination into the source and ionic composition of the accumulating fluid. Table VI reveals that the cationic composition of samples taken directly from steadily accumulating resevoirs in the inner corner is slightly hypertonic with respect to plasma Na^+, but several fold hypertonic to plasma K^+ concentrations. The tear, nasal drip, and plasma samples analyzed and presented in this table were taken from birds prior to their use in the isotope studies. Accumulations of widely intermittant and overflowing tear-like secretions, which encrusted the feathers were observed only among ducks given salt water for drinking. Samples of tears of fresh water ducks displayed similar ionic compositions. A further examination into the source of this fluid from the eye of salt water ducks revealed a noticeable hypertrophy of the Harderian or Harder's gland. Table VII presents a comparison of the absolute and corrected wet and dry weights of these glands, and provides a contrasting comparison of salt diet effects on kidney and salt gland weights. The wet and dry weights of salt glands were observed to increase in response to continuous high salt intake, and appear in agreement with previously documented increases reported for glands in both ducks and seagulls (Benson and Phillips 1964; ? Holmes, Buttler and Phillips 1961). The corrected weights of Harderian glands were also observed to be significantly ($p = < 0.001$) larger in salt fed birds while there was no apparent treatment effect on corrected kidney size ($p = < 0.15$).

DISCUSSION

Compartment Volumes in Non-Stressed Birds

A comparison of the pre-salt load sizes of Br^{82}, H_2^3O and T-1824 spaces among the three types of birds tested indicates that relative to body weight the volumes for these spaces are noticeably larger in ducks and gulls than in roosters. Support for the observed differences in plasma volume is given by the consistency of the data in Tables I and II and previously published determinations for two of the groups tested. Measurements in two White Leghorn roosters ($\bar{x}$ = 4.4 gm % body weight) appear in agreement with determinations reported by Bond and Gilbert (1958) for comparably sized birds (3.1 ± 0.4%). Similarly, values for fresh water reared Pekin ducks ($\bar{x}$ = 6.± 0.3 gm % body weight) largely substantiate those of Portman et al. (1952) for White Pekin ducks of the same size ($\bar{x}$ = 5.5 ± 0.12%). Although there are presently no other published estimates of plasma volume in gulls the reported percent body weight ($\bar{x}$ = 7.16%) agrees closely with similar measurements on other species frequenting marine environments such as the redhead and canvasback ducks (7.1 ± 0.2%; Bond and Gilbert, 1958). The distinct differences in plasma volume-body weight proportions observed between roosters on one hand and ducks and gulls on the other, corroborate the findings of Bond and Gilbert (1958) which showed a comparable difference between a large variety of aquatic and nonaquatic birds.

In an investigation of the structural and functional adaptations of diving ducks, Bond and Gilbert (1958) have shown that the principal difference in oxygen carrying capacity between aquatic birds, which commonly have functional nasal glands, and non-aquatic birds, which either lack or have atrophied glands, is a substantially larger blood volume (greater number of red blood cells and greater plasma volume) in the former. The blood volumes % body weight observed in this study for fresh water ducks (10.6%), gulls (14.6%), and roosters (8.1%) are consistent with the previously mentioned plasma volume differences, and together support the observations of Bond and Gilbert.

The additional finding that greater vascular volumes in the aquatic birds are accompanied by greater total body water may indicate other functional significances for the greater availability of body water. The presence of a larger volume of body water relative to total body mass would provide the advantage of a greater ability to withstand dehydrating conditions or sudden extracellular osmotic increases. Bond and Gilbert have shown that there is a greater vascular volume among birds that are stronger fliers, which is characteristic of most aquatic species. The presence of larger H_2^3O spaces in conjunction with increased plasma volumes could well function to compensate for large evaporative losses incurred during increased physical activity or to minimize ionic changes in extracellular fluids resulting from the ingestion of hypertonic food or drink. The hypertonic secretory capacity of the nasal glands could also serve as an adaptation particularly suited for ion regulation under dehydrating conditions such as prolonged flight or the ingestion of sea water.

Response to Hypertonic NaCl Injection

An equivalent amount of NaCl per unit body weight injected into seagulls, fresh and salt water ducks, and roosters produced noticeable differences in the extent of Br^{82} space change and in the amount of sodium eliminated by each group. The response of the roosters was marked by a larger influx of water into the extracellular Br^{82} space followed by renal filtration of about 20% of the salt load. Fresh water ducks (C-3#6 and C-3#1), in contrast, demonstrated a greater capacity to absorb this load without intercompartmental water fluxes. The magnitude of Br^{82} space changes observed in post-salt load gulls, salt water ducks and secreting fresh water ducks closely resembled the response of the roosters.

In all of these birds which exhibited a greater redistribution of body water and an increase in extracellular volume the changes in Br^{82} space appeared to parallel the onset of osmoregulatory activity. Although no attempt was made to establish the relationship or temporal sequence of secretion or excretion and compartment changes, it would appear reasonable that the function of specialized osmoregulatory organs may reflect a limit of acommodation to osmotic changes on the part of the organism. The more energy conservative process of body water redistribution may correlate with the organisms ability to respond to osmotic stress by renal and extrarenal mechanisms. If so, a maximum in intercompartmental accommodation might be predicted to precede the specialized functioning of nasal glands and kidneys. The occurrence of significantly larger dilutions of extracellular volume in those birds which responded to the injected load by these mechanisms supports this idea.

The greater capacity of non-secreting fresh water ducks to absorb the salt load without secretion or elimination is further evident in post-salt load plasma sodium measurements. Inspite of the retention of a major portion of the salt load fresh water ducks, without nasal gland secretion, showed a smaller average increase in plasma sodium concentration (32 mEq/l) than did secreting fresh water ducks (41 mEq/l) or salt water ducks (45 mEq/l). Lower post-salt load levels of plasma Na^+ together with reduced expansion of the extracellular space suggest a more rapid and complete distribution of the injected load in these birds. The disappearance of injected sodium from the vascular space into interstitial, lymphatic and intracellular spaces, in the absence of a net flow of body water into the extracellular (Br^{82}) space, would suggest a greater ability of the cell mass of non-secreting fresh water ducks to absorb an increase in sodium concentration.

Since neither relative body water, compartment sizes nor plasma Na^+ and K^+ concentrations appeared significantly different between the fresh and salt water ducks, it would seem that the differences in compartment responses were not due to salt load effects on these parameters. Individual or species response differences observed in these studies might also be due to variables which include dehydration tolerance, excretory capacity or threshold and hormonal state.

Although the above observations might serve to implicate ECF volume increases in nasal gland secretion, the exact nature of the stimulus which triggers secretion by the salt gland remains unresolved. The original descriptions of the nasal gland as a significant osmoregulatory organ in a variety of marine birds by K. Schmidt-Knielson and co-workers (1958) revealed that secretion can be induced by non-specific increases in plasma osmotic concentration and that neuronal stimulation proceeds via parasympathetic ennervation. Endocrine studies by Holmes et al. (1961) have shown that obliteration of the extrarenal response in adrenalectomized ducks could be reversed by the injection of cortisol, corticosterone, cortexone, or aldosterone prior to salt loading. Furthermore, it was shown that administration of any of the above hormones or adrenocorticotrophic hormone (ACTH) prior to salt loading in normal birds resulted in increases in the initial flow rate, volume, and Na^+ and K^+ concentrations of the drip. Although there was no demonstration of a direct effect of these endocrines on gland activity, their presence was established as necessary for some undefined sequence of events leading to nasal secretion. In a brief description of the results from an undetailed experiment, Holmes (1965) reported measuring an increase in blood volume prior to the initiation of secretion in a salt-loaded duck. Injection of an equal volume of gum arabic was also reported to have stimulated secretion. Holmes concluded that a volume receptor rather than a baroreceptor or osmoreceptor participates in the stimulating mechanism.

More recently Hajjar *et al.* (1970) investigated the significance of osmolarity in the stimulation of secretion in western gulls (*Larus occidentalis*). Their findings indicated that only hypertonic intravenous infusions (5% NaCl, 27.5% sucrose, 10% mannitol) were effective in eliciting secretion, and no secretion was produced with either hypotonic sodium solutions or hypotonic blood volume expansion (6% Dextran 70 in 0.9% NaCl). Similar conclusions were reached by Ash (1969) working with Aylesbury ducks. Administration of hypertonic sucrose, mannitol, or sufficient NaCl to raise plasma osmolarity 2-8% elicited secretion in these birds. Since intravenous injection of hypertonic KCl, urea, or dextrose failed to evoke secretion Ash concludes that permeability of the hypertonic solute is also important in the secretory mechanism.

Although there are presently no explanations that link endocrine, osmotic, and other factors to nasal gland secretion, there is evidence which appears to offer a plausible connection which bears on the results of this study. Experiments with dogs have shown that increases in extracellular fluid volume produced by isotonic expansion of the space stimulate the release of antidiuretic hormone (ADH) and aldosterone which function to conserve urinary water and sodium losses and further augment expansion of the extracellular volume (Bartter, 1963). The stimulation of adrenal activity might also lead to indirect effects on ECF volume such as that produced by corticoids, like cortisone, which has been shown to increase plasma volume and the extracellular space in humans (Bartter, 1963). The presence of volume or stretch receptors which would monitor extracellular space changes could serve as the transducers of osmotic and neural stimuli for nasal gland and other compensatory mechanisms. Although the report by Holmes of secretion accompanying vascular volume change constitutes the only previous evidence for a volume type receptor, the coincidence of a large Br^{82} space volume change with the presence of nasal gland activity in this study lends further support to the notion of such a receptor. Hematocrit changes, as previously mentioned, appeared unrelated to gland activity initiated by salt loading. This would suggest that receptors, if present, are responsive to extravascular volume change.

Descriptive and Functional Aspects of Compartment Spaces

Critical to interpretation of the significance of tracer dilution spaces is both the nature of the volume penetrated by the marker substance and the relevance of this space to the size and behavior of the intended body compartment. The conceptualized divisions of body fluid spaces into intracellular and extracellular compartments which definitionally distinguish intracellular water content from that of the circulating and rapidly exchanging intra- and extravascular spaces, have been difficult parameters to define accurately by experimental methods. The absence of tracer substances which are limited to the domain of any one compartment or subcompartment, and inability to accurately measure the error contributed by impenetrability, metabolic degradation, or non-specific binding remain the principal difficulties of the tracer technique.

Estimates of total body water, from the dilution space of tritium oxide, have been demonstrated to be among the most reliable of the tracer methods. Reid et al. (1958) have compared water content of rabbits as measured by tritium oxide, antipyrene, and N-acetyl-4-aminoantipyrene dilution and have demonstrated coefficients of correlation approaching unity for each (0.98, 0.99, 0.99 respectively) when compared to estimates by dessication. The principal errors of H_2^3O estimates lie in tritium exchanges with hydrogens other than those of water, and equilibration with osmotically isolated intestinal water. The exchange of tritium for hydrogen in hydrocarbons and other hydrogenated compounds has been shown to be a relatively slow process in comparison to body water dilution, and has been attributed to account for a 0.5 - 2.0% over-estimation of this space relative to body weight in humans. (Prentice et al. 1952). H_2^3O has been shown also to exchange with gut water. Bidirectional measurements have shown this exchange to be an irregular forced-flow like process with complete labelling of intestinal fluid taking somewhat longer than that of the intracellular and extracellular volumes (Visscher et al. 1944). The general reliability of tritium measurements are further substantiated by the similarity of total body water estimates for roosters in these experiments (54.3% body weight) and those reported by

Medway and Kare (1959; 53.3% body weight) using antipyrene, a tracer substance which has been shown by others to yield nearly identical estimates.

The substantially different H^3_2O spaces among the three birds measured may in part be due to equilibration differences mentioned above or to differences in the proportions and types of body tissues, but other evidence indicates a fundamental difference in this parameter among the three species tested. Support for this conclusion comes from the average hematocrits (Table III) which, for all the birds receiving fresh water, appeared in close agreement with previously published averages for comparable sized roosters (45.0 ± 2.0%; Sturkie, 1965), ducks (43.0 ± 2.0%; Bond and Gilbert, 1958), and gulls (46.0 ± 1.2%; Hughes, 1970). This would tend to discount the possibility of any major differences in their states of hydration, at least to the extent necessary to account for the relatively large H^3_2O space variances. Evidence too, of the reproducibility of the technique and the agreement of its results in one case with those of other investigators mentioned above, further strengthen this conclusion. The insignificant, but consistent differences between H^3_2O space as a percent of body weight in fresh and salt water ducks (Table I) probably reflect a dehydrating effect coupled to a stress induced storage-tissue deficit resulting from increased NaCl intake (Krista _et al_. 1961; Holmes _et al_. 1961).

The nature of the calculated intracellular fluid volume decrease which closely mirrored the increase of the Br^{82} space suggests a precursor-product relationship which is consistent with known properties of animal tissues for regulating osmotic equilibrium through shifts in intracellular ions and/or water. The reciprocal relationship between these two changes reflects what can be seen in Table III to be a relatively unaffected H_2^3O space response to osmotic stress. The fluctuations and generally small increases in H_2^3O space following salt injection are thought to reflect either the error inherent in the technique (SE = 2.3 to 4.1% for H_2^3O space measurements in control ducks without salt loads), or possible shifts of body water from more slowly labelled compartments, such as the intestinal tract, which would serve to dilute the pre-existing isotope space. The 1.0 - 1.5 hours permitted for equilibration of tritium label in animals of this size was thought sufficient for exchange with most physiological water since similar labelling studies have shown that 1-2 hours produces uniform labelling of body water in humans (Moore et al. 1963). Little information exists, however, on the equilibration rate of H_2^3O with anatomically sequestered water such as that of the stomach, bladder, or intestine.

The use of tracer substances to measure accurately extracellular water volume has been hampered both by the lack of a tracer with sufficient specificity for this volume, and by uncertainties about the functional and anatomical boundaries of this space. The definition of extracellular space as described by Bernard (1920) and more recently by Manery (1954) subdivides this space into circulating fluids (blood and lymph) and stationary fluids (interstitial, cerebrospinal, pleural, pericardial, peritoneal and synovial fluids, and aqueous and vitreous humor). The physiological significance of these subdivisions for the extracellular storage, exchange and movement of ions and water is largely unknown. Methods for measuring total extracellular space include substances such as sucrose and inulin which because of their size are slow to diffuse with the result that equilibrium times are long and thereby estimates are subject to errors of excretion and metabolism. The smaller substances of thiosulfate and radiosulfate diffuse more rapidly, but are actively metabolized by the kidneys. Since they lack compartmental selectivity and the full extent of their equilibration is unknown, use of these substances is of questionable significance (Berson and Yalow, 1955). The radioisotopes Na^{24} and Br^{82} appear to be more reliable in these regards since both are rapidly diffusing species with largely extracellular domains during the time of their equilibration and both are only normally excreted. The overestimation of ECF volumes by these ions due to exchange with intracellular sodium or chloride have been found to be measurable by simultaneous measurements of red blood cell penetration. These corrected Na^{24} and Br^{82} extracellular space estimates in humans (18 - 26% body weight) appear in general agreement with the lengthier inulin measurements (13 - 19% body weight; Rovner and Conn, 1963). ECF volume estimates for roosters (28.8% body weight) obtained by the Br^{82} method in these studies agreed with results of the thiocyanate method in White Leghorn chickens (26.2%) as determined by Medway and Kare (1959).

The nature of the Br^{82} space changes observed in these experiments are thought to represent a predominance of dilution by intracellular water but may also reflect an influx of Br^{82} under rapidly changing osmotic conditions. There were no attempts to measure red blood cell permeation under various experimental conditions. The demonstration of a rapid and measurable change in Br^{82} space in response to osmotic stress, however, illustrates the significance of ion and water movement in accommodation to sudden osmotic change. A possible significance for this volume change as a predictor of regulatory responses or as an indicator of the osmotic state of the organism appears worthy of further examination.

Sodium Elimination

The observation of more active nasal glands producing a highly concentrated secretion in gulls is consistent with published data which indicates this same constituitive superiority in glands from a variety of gulls (Schmidt-Nielsen, 1964) as compared to either wild Mallard or domesticated Pekin ducks (Schmidt-Nielsen and Kim, 1964). The large differences observed between total nasal gland Na^+ secretion in fresh and salt water ducks (Table IV) can be attributed in part to a volume difference resulting from what has been demonstrated to be a state of glandular hypertrophy in salt acclimated ducks (Benson and Phillips, 1964). The negative response to the salt load witnessed in a number of fresh water ducks as well as the small percentage of Na^+ eliminated by the nasal gland in the remainder of this group suggest, however, that the principle difference is due to the absence or cessation of secretory stimuli in these fresh water birds. The collection of drip with Na^+ concentrations comparable to those from salt water ducks (600 mEq/l), and the observation of drip in response to a second salt load at the end of the experiment in those birds not responding to the initial load, confirmed the presence of active or potentially active salt glands in all of the fresh water ducks.

Renal elimination of the injected sodium appeared to be a far less efficient mechanism for maintaining body water while removing excess salt in ducks and gulls. Table V reports post-salt load in concentrations for plasma nasal gland secretion, and cloacal fluid in representative birds from each group. The hypotonic cloacal fluid sodium concentrations under conditions of highly elevated plasma Na^+ levels (Figures 1 - 5), are consistent with previous unsuccessful efforts by Douglas (1970) and Hughes (1970) to obtain the significantly hypertonic urine Na^+ concentrations which are reportedly possible for the duck (600 mEq/l) and gull (300 mEq/l) kidneys (Holmes et al. 1961). The relatively low percentage of injected sodium which was either secreted or excreted by fresh water ducks, as opposed to the other three groups of birds, clearly represents a greater capacity to redistribute this osmotic load and to accommodate through intercompartmental change.

Eye-Fluid and Gland Weights

The observation reported here of the accumulation of hyperkalemic fluid in the eye region of ducks which had been acclimated to high salt intake, offers the interesting possibility that this fluid may be a product of active ion elimination. Although the conditions under which the secretion was observed did not permit distinguishing this as a controlled physiological response the injection of hypertonic NaCl was observed to increase the amount of fluid present in the inner canthus. Such secretion could also be a nonspecific cholinergic response in conjunction with increased parasympathetic stimulation of the nasal glands. The accumulating fluid induced by salt injection was observed to fall free of the eye in the gulls and fresh water ducks onto feathers which appeared well oiled and water repellent. Accumulations in the eyes of saline fed ducks appeared, in contrast, to drain into the surrounding feather region. Prolonged secretion of this fluid which is 5 - 25 times more concentrated than plasma K^+ could result in a significant elimination of this ion.

Attempts to identify the source of this secretion have implicated the Harderian gland, which is increased in size in birds exhibiting exterior accumulations of dried fluid. Preliminary investigations by Hughes (unpublished observations) has revealed a large duct which extends from this gland dorsad along the orbit and opens immediately above the eyeball.

Although the coincidence of larger Harderian glands in the salt fed ducks can only be considered circumstantial evidence for their association with excess eye fluid, the presence of these glands as the only conspicuously altered tissues of that region in salt water fed ducks warrants future confirming investigation. Histological staining of tissues from salt and fresh water ducks has shown that ducts and apocrine cells from the enlarged glands are richer in alkaline phosphatase. Each of these observations is presented only as preliminary evidence from work secondary to the aims of this study.

SUMMARY

1. Estimates of plasma volume, extracellular fluid (ECF) volume and total body water (TBW) in fresh and NaCl water reared Pekin ducks, Glaucous-winged gulls, and White Leghorn roosters revealed greater plasma volumes relative to both total body water and body weight in ducks and gulls. Extracellular fluid volume estimates revealed substantially larger spaces relative to total body water in roosters as opposed to gulls and ducks. In contrast, TBW was considerably larger in ducks and gulls as opposed to roosters. Saline feeding produced no significant differences in compartment sizes relative to body weight or TBW in Pekin ducks. Generally larger average plasma volumes and lower hematocrits, however, were observed in NaCl reared ducks.

2. The effects of an intravenous injection of hypertonic NaCl solution were observed on the intercompartmental distribution of body water. Expansion of the extracellular space in response to salt loading was seen to be greatest in gulls, salt acclimitized ducks, and fresh water ducks exhibiting nasal gland activity. Fresh water reared ducks generally showed a greater tolerance for hypertonic NaCl loading as evidence by a smaller change in ECF volume and little or no nasal gland or kidney elimination of the load. In contrast, saline fed ducks

exhibited marked changes in ECF volume spaces together with sizable amounts of nasal drip after salt loading. The nature of this response closely resembled those of the gulls. Redistribution of total body water is implicated as part of osmoregulatory stimuli.

3. The extent of sodium removal by nasal glands and kidneys is compared among birds receiving salt loads. The salt ducks and gulls, which exhibited nasal gland secretion, voided a major portion of their salt load via this route while the renal mechanism in both salt and fresh water ducks accounted for only a small source of elimination. The efficiencies of the kidney and nasal glands in removing the initial salt load are compared using isotopically labelled $Na^{22}Cl$.

4. A preliminary report is made on the ionic composition of excess eye secretion observed accumulating on the feathers below the inner canthus in NaCl acclimitized ducks. Implications of the Harderian glands as a possible source of this fluid are made from gland weight comparisons in fresh and salt water ducks.

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TABLE I. Comparisons of plasma volume (T-1824 Space), extracellular fluid volume (Br^{82} Space), and total body water (H_2^3O Space) in fresh and NaCl (0.483 N) water acclimated ducks (Anas platyrhynchos), gulls (Larus glaucescens), and White Leghorn roosters (Gallus domesticus). Hematocrit values represent means of individual values from 2 - 3 pre-salt load blood samples. The sample number in each measurement is indicated in ( ).

a = Significant difference ($p = < 0.01$)

b = No significant difference ($p = < 0.40$)

BIRDS	Body Wt. (gm.)	Hemat.	T-1824 Space (ml.)	Br^{82} Space (ml.)	H_2^3O Space (ml.)
fresh water ducks	3091.2[a] ±148.15 (7)	0.434[b] ±0.010 (7)	185.4 ±8.2 (11)	765.7 ±38.2 (7)	2120.6 ±108.9 (11)
salt water ducks	2352.8[a] ±74.0 (7)	0.417[b] ±0.012 (7)	158.6 ±5.1 (12)	623.4 ±42.0 (7)	1512.2 ±76.2 (7)
gulls	835.1 ±13.2 (2)	0.512 ±0.078 (2)	59.9 ±2.4 (2)	321.3 ±31.1 (2)	734.6 ±73.9 (2)
roosters	2506.0 ±110.9 (2)	0.479 ±0.002 (2)	106.2 ±1.3 (2)	724.5 ±99.4 (2)	1362.6 ±89.3 (2)

TABLE II. Plasma volumes (T-1824 Space) and extra-cellular fluid volumes (Br^{82} Space) corrected for body weight and total body water; total body water ($\dot{H}_2^3O$ Space) corrected for body weight. The standardized compartment values are expressed as either % body weight or % H_2^3O Space. The sample number in each case are those listed in Table I.

a = Significant difference ($p = < 0.05$)

b = No significant difference

BIRDS	T-1824 SPACE		Br^{82} SPACE		H_2^3O SPACE
	% body wt,	% H_2^3O space	% body wt.	% H_2^3O space	% body wt.
fresh water ducks	5.75[a] ±0.16	9.19[b] ±0.48	24.88[b] ±1.00	36.35[b] ±1.58	68.54[b] ±0.84
salt water ducks	6.49[a] ±0.27	10.59[b] ±0.52	26.43[b] ±1.38	41.31[b] ±2.02	63.97[b] ±2.66
gulls	7.16 ±0.17	8.14 ±0.23	38.24 ±3.10	43.64 ±2.95	87.94 ±1.14
roosters	4.43 ±0.32	7.81 ±0.35	28.79 ±2.66	52.92 ±3.81	54.32 ±1.09

TABLE III. Changes in Br^{82} and H_2^3O Spaces following intravenous injection of 0.2% body weight (v/w) of 5 N NaCl. Maximum post-salt load Br^{82} Spaces represent the largest volumes observed during the 60 minute period following salt injection. % change in Br^{82} Space represents the magnitude of change relative to the mean pre-salt load volume. Recorded too are the volumes of collected nasal gland secretion where present. Mean pre- and post-salt load Br^{82} Spaces are given with their standard errors.

a = Significant difference ($p = < 0.01$)

	Mean Preload Br^{82} Space (ml.)	Max. Postload Br^{82} Space (ml.)	Max. Change in Br^{82} Space (ml.)	% Change Br^{82} Space	Nasal Gland Secretion (ml.)	Mean Preload H_2^3O Space (ml.)	Mean Postload H_2^3O Space (ml.)
FRESH WATER DUCKS							
C-6#2	828.4 ± 19.1					1960.0 ± 37.1	
C-6#1	650.9 ± 23.8					2017.0 ± 59.3	
C-3#6	706.0 ± 21.6	757.6	51.6	7.30	none	2163.7 ± 30.5	2206.6±68.0
C-6#3	841.3 ± 0.4	1003.7	162.4	19.30	<0.3	2480.3 ± 12.2	2593.4±69.7
C-3#3	922.9 ± 46.3	1176.4	183.5	18.48	1.26	2382.4 ± 35.3	2407.3±33.2
C-6#5	743.7 ± 32.1	787.2	43.5	5.82	none	2221.7 ± 68.9	2248.6±29.6
C-3#1	666.7 ± 26.1	701.8	35.1	2.07	none	1618.8 ± 51.3	1575.1±21.3
$\bar{X}$				10.59 [a]			
±SE				± 3.49			
SALT WATER DUCKS							
C-1#4	573.7 ± 17.6					1655.7 ± 35.9	
C-4#5	607.3 ± 25.7					1631.9 ± 30.5	
C-1#1	621.8 ± 3.8	772.5	150,7	24.25	20.82	1316.1 ± 1.4	1375.9±32.5
C-2#2	768.3 ± 35.9	977.2	208.9	27.20	2.43	1576.4 ± 47.5	1624.0±30.9
C-3#4	776.9 ± 7.8	931.7	154.8	19.92	5.18	1770.6 ± 47.8	1913.8±59.9
C-3#3	515.4 ± 18.1	691.9	176.5	34.25	5.17	1193.7 ± 51.3	1273.8±51.1
C-2#1	500.1 ± 4.8	609.4	109.3	21.87	34.25	1395.9 ± 5.5	1457.3±37.2
$\bar{X}$				25.49 [a]			
±SE				± 2.50			
GULLS							
G-1	352.3 ± 6.7	451.1	98.8	28.04	6.14	756.1 ± 7.1	751.6±17.7
G-2	290.2 ± 5.4	400.9	110.7	38.15	5.95	713.1 ± 5.9	714.9± 9.4
$\bar{X}$				33.09			
±SE				± 7.14			
ROOSTERS							
R-1	625.1 ± 11.7	725.7	100.6	16.10	-	1273.3±11.4	1451.9±19.8
R-2	823.8 ± 5.3	968.4	144.4	17.54	-	1277.8±31.1	1483.6±27.1
$\bar{X}$				16.82			
±SE				± 1.01			

TABLE IV. Sodium elimination via cloacal fluid and nasal gland secretion following intravenous injection of 5N NaCl (0.2% body weight) in fresh and NaCl (0.483N) water fed ducks (*Anas platyrhynchos*), Glaucous-winged gulls (*Larus glaucescens*), and White Leghorn roosters (*Gallus domesticus*). In those birds which received trace amounts of $Na^{22}Cl$ (5 - 15 mC) the isotopically labelled salt was administered together with the 5 N NaCl salt load. Means are given together with their standard errors.

	mEq. Na^+ injected	CLOACAL EXCRETION		NASAL GLAND SECRETION		INJECTED Na^{22}	Na^{22} in CLOACAL EXCRETION		Na^{22} in NASAL GLAND SECRETION	
		mEq.Na^+	% of Na^+ injected	mEq.Na^+	% of Na^+ injected	cpm x 10^{-7}	cpm x10^{-4}	% Na^{22} injected	cpm x 10^{-4}	% Na^{22} injected
FRESH WATER DUCKS										
C-6 #4	31.00	1.26	4.05	0.135	0.44					
C-3 #4	20.00	1.11	5.54	0.104	0.52	1.276	19.12	1.49	NO	DRIP
C-3 #6	30.00	0.17	0.58	0.000	0.00					
C-6 #3	36.05	0.86	2.38	0.000	0.00					
C-3 #3	35.25	0.44	1.26	1.065	3.67	1.306	8.09	0.62	4.72	0.36
C-6 #5	29.00	1.63	5.62	0.000	0.00	1.463	20.86	1.42	NO	DRIP
C-3 #1	30.00	0.92	3.06	0.000	0.00					
$\bar{X}$			3.21		1.54					
± SE			±0.75		±0.58					
SALT WATER DUCKS										
C-4 #1	26.00	0.000	0.00	4.182	16.08					
C-5 #1	26.50	2.172	8.19	14.538	54.86					
C-1 #1	23.00	0.482	2.09	11.868	51.60	1.331	NO	FLUID	188.88	14.19
C-2 #4	24.50	0.694	2.83	1.446	5.96					
C-5 #4	26.50	0.235	0.88	2.934	11.07					
C-5 #3	25.00	0.473	1.89	2.980	11.93					
C-2 #2	27.50	0.833	3.03	18.260	66.40	1.571	12.97	0.83	217.87	13.87
$\bar{X}$			3.15		31.12					
± SE			± 0.97		±9.59					
GULLS										
G-1	8.50	0.114	1.34	5.095	59.94	0.873	48.25	5.50	114.11	15.18
G-2	8.00	2.049	25.61	5.081	63.51	0.851	1.35	0.10	136.24	15.90
$\bar{X}$ ±SE			13.47±12.		62.22±1.8					
ROOSTERS										
R-1	22.00	5.194	23.61	-	-					
R-2	27.00	3.198	11.85	-	-	0.479	70.67	14.70	-	-
R-3	21.50	4.603	21.41	-	-					
$\bar{X}$ ± SE			18.96±4.4							

TABLE V. Electrolyte concentrations of simultaneously sampled cloacal fluid, nasal gland secretion and plasma at intervals prior to and following the injection of 0.2% body weight (v/w) of 5 N NaCl. Ion concentrations are given as mEq/l.

	Sample Time min. - before + after salt loading	Cloacal Fluid			Nasal Gland Secretion			Plasma Concentration	
		Volume (ml.)	Concentration Na$^+$	Cl$^-$	Volume (ml.)	Concentration Na$^+$	Cl	Na$^+$	Cl$^-$
FRESH WATER DUCKS									
C-3 #6	-45	7.17	23.0	15.2				136.2	101.3
	+120	7.53	131.2	113.3				158.2	145.4
C-6 #5	+42	2.51	77.1	57.4				173.4	170.8
	+120	13.42	107.0	80.0				162.9	140.9
SALT WATER DUCKS									
C-1 # 1	- 100	6.65	68.5	55.4				155.4	-
	- 60	6.72	39.1	44.8				146.9	-
	+ 20				7.24	592.0	594.9	151.7	138.4
	+ 40				6.13	602.0	606.5	157.9	-
	+ 60				5.06	600.0	594.9	155.0	136.3
	+ 90				2.40	569.6	585.4	153.6	134.9
	+ 120	6.00	80.2	65.4				152.2	129.4
GULL									
G-1	- 65	3.43	89.1	46.2				141.8	118.0
	- 25	4.59	100.5	58.0				140.4	126.7
	+ 82				5.90	817.3		169.1	144.4
	+ 122				0.25	890.3		147.8	120.8
	+ 130	6.65	164.5	160.4				150.4	123.8
ROOSTER R-1	+ 4	12.40	74.1	32.4				154.2	-
	+ 75	10.97	163.7	168.0				156.8	-
	+ 130	13.83	178.7	127.2				169.6	-

TABLE VI. Electrolyte concentrations of eye fluid relative to plasma and spontaneous nasal gland secretion in ducks (*Anas platyrhynchos*) reared on fresh water and NaCl (0.483N) solution. Concurently produced samples were taken during the 2 - 3 week period prior to the terminal experiments. Ion concentrations are given as mEq/l.

	EYE FLUID			PLASMA			SPONTANEOUS NASAL GLAND SECRETION		
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	Na^+	K^+	Cl^+
SALT WATER DUCKS									
C-5 #3	276.0	29.2	255.0	178.4	4.6	125.0	192.0	6.6	140.4
C-2 #3	196.5	40.0		161.2	4.1	119.0			
C-2 #4	134.8	49.2		145.6	4.0	106.5			
C-4 #6	112.0	98.4		162.8	3.6	114.8			
C-5 #6	208.0	34.4		158.6	3.7	113.0			
C-5 #5	176.4	36.0		151.0	3.8	108.0			
C-5 #1	136.8	27.6		146.0	2.9	104.0	300.0	11.2	
C-4 #4	154.0	72.8		143.6	4.6		346.0	12.0	
C-1 #1	145.2	27.2		144.2	3.4	106.8	400.0	11.0	
C-4 #3	136.8	16.8		146.4	4.2		414.4	16.0	
FRESH WATER DUCKS									
C-6 #1	150.8	28.0		155.8	3.9				
C-6 #2	129.2	24.0		157.0	4.6				
C-6 #3	106.0	26.4		149.8	4.8				
C-6 #4	136.8	40.0		155.8	3.7				
C-6 #5	136.8	33.6		155.0	3.4				

TABLE VII. Absolute and corrected weights of Harderian glands, nasal glands, and kidneys from fresh water and NaCl (0.483N) water reared Pekin ducks (Anas platyrhynchos). Means are given together with their standard errors. Sample sizes are indicated by the numbers in ( ).

a = Significant difference ($p = < 0.001$)

b = Significant difference ($p = < 0.001$)

DUCKS	Harderian Glands			Nasal Glands			Kidneys		
	Dry Wt.	Wet.Wt	mg.% Bdy.Wt.	Dry Wt.	Wet Wt.	mg.% Bdy.Wt.	Dry Wt.	Wet Wt.	mg.% Bdy. Wt.
	grams			grams			grams		
fresh water (11)	0.213 ±0.024	0.982 ±0.087	33.29[a] ±2.82	0.019 ±0.005	0.318 ±0.017	10.93[b] ±0.55	4.23 ±0,39	19.16 ±1.84	0.644 ±0.054
salt water (24)	0.269 ±0.013	1.190 ±0.056	51.16[a] ±2.53	0.252 ±0.011	1.047 ±0.051	44.91[b] ±2.24	4.03 ±0.14	18.36 ±0.65	0.798 ±0.045

FIGURE 1. Changes in fluid compartment volumes and plasma Na$^+$ concentration in response to salt loading in a fresh water fed duck, Anas platyrhynchos, which failed to produce nasal gland secretion. 0.2% body weight (v/w) of a 5 N NaCl solution was injected intravenously at the time indicated by the vertical line. Effects of the salt load were observed in the following compartments: Graph① ECF volume (Br^{82} Space); Graph② Total body water (H^3_2O Space); Graph③ Intracellular volume (H^3_2O Space - B^{82} Space); Graph④ Plasma Na$^+$ concentration (mEq/l).

□————□ Salt loaded duck C-3 #1.

●— — —● Control fresh water duck C-6 #2; no salt load.

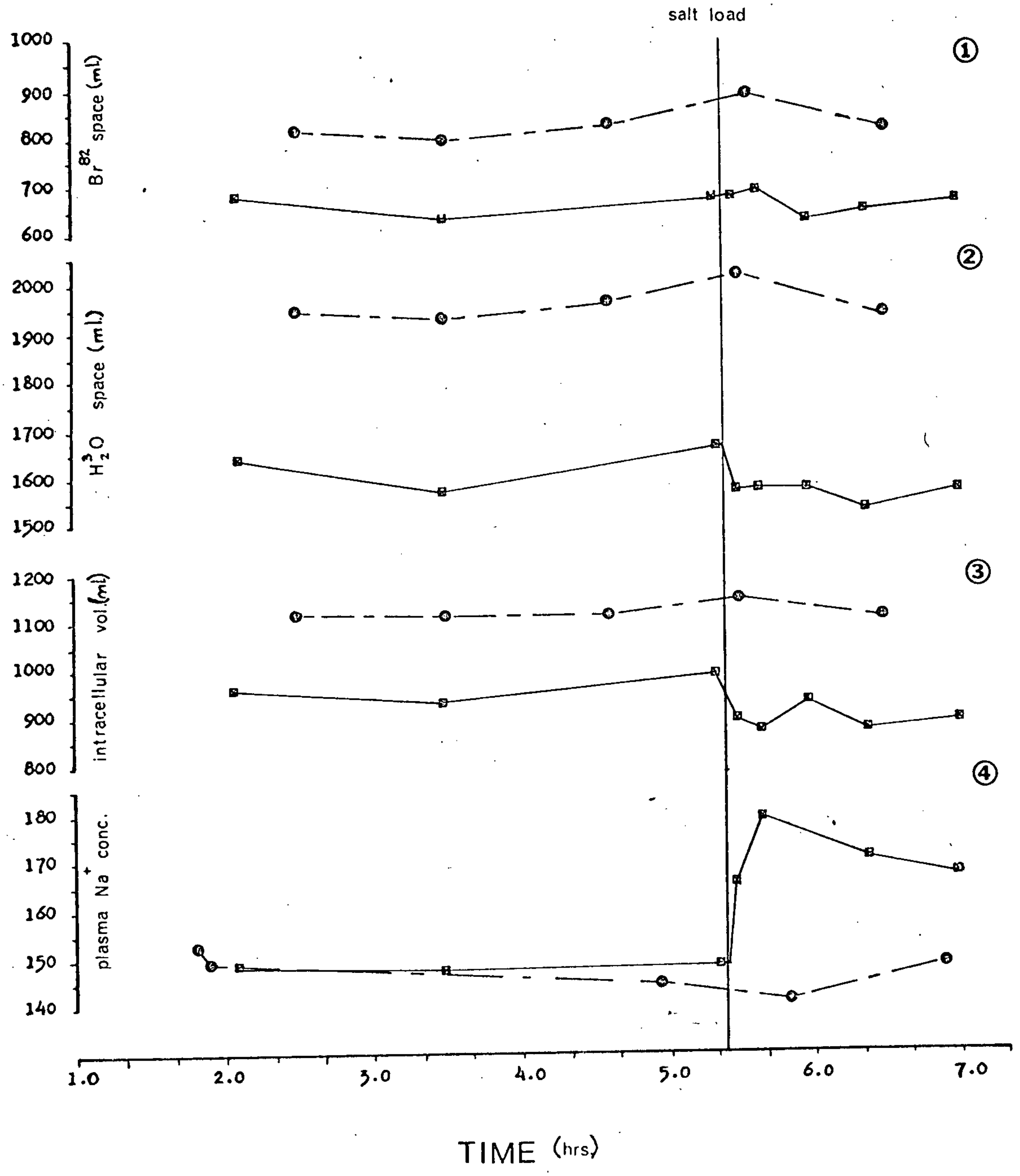

salt load
①
②
③
④
Br^{82} space (ml)
1000
900
800
700
600
H_2^3O space (ml)
2000
1900
1800
1700
1600
1500
intracellular vol.(ml)
1200
1100
1000
900
800
plasma Na^+ conc.
180
170
160
150
140
1.0
2.0
3.0
4.0
5.0
6.0
7.0
TIME (hrs)

FIGURE 2. Changes in fluid compartment volumes and plasma Na^+ concentration in response to salt loading in a fresh water fed duck, Anas platyrhynchos, which demonstrated nasal gland secretion. Conditions of the salt load and coordinates of the measured responses are the same as those described in Figure 1.

○————○	Salt loaded duck C-3 #3.
●— — —●	Control fresh water duck C-6 #2; no salt load.

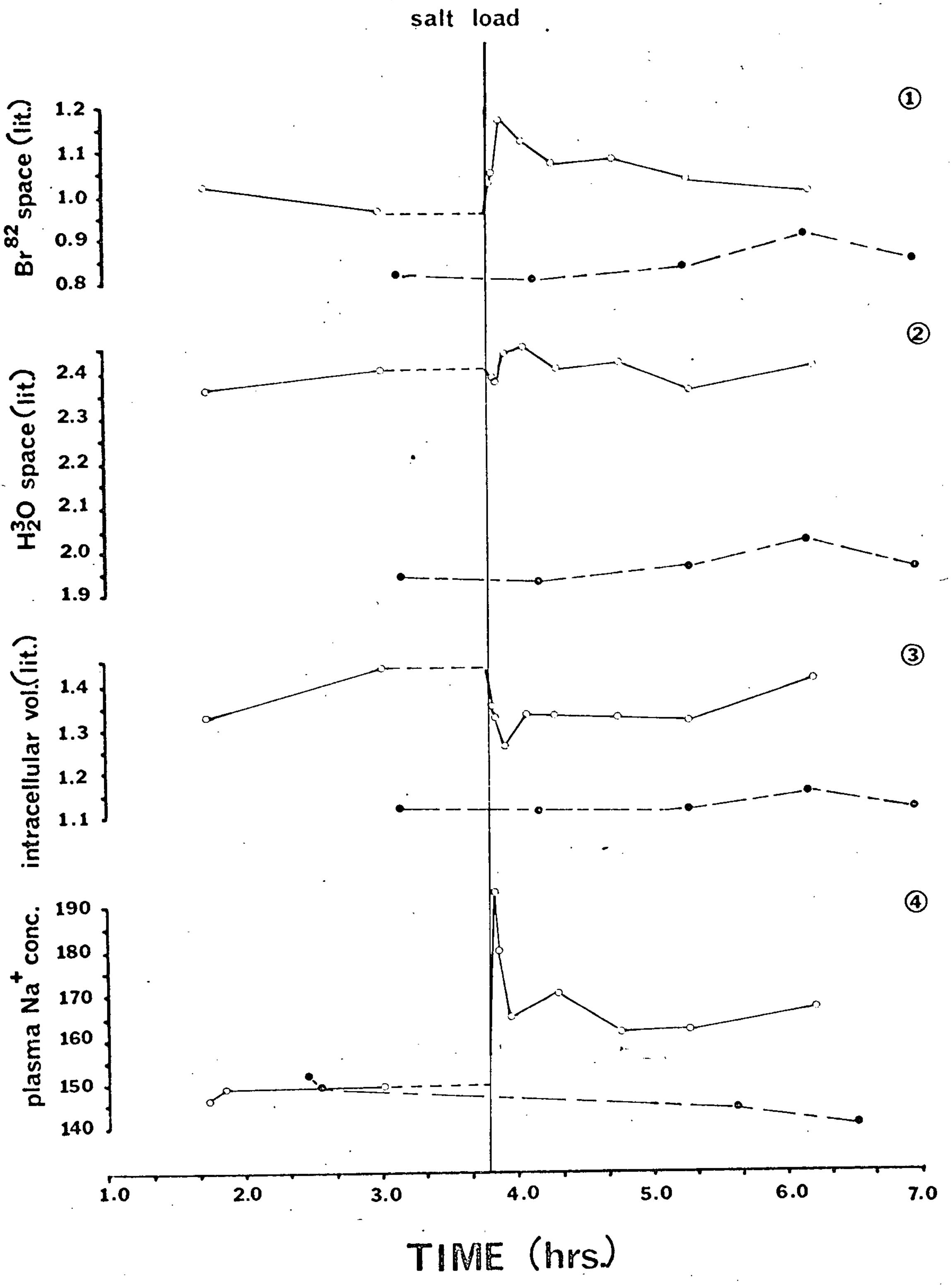

salt load
①
②
③
④
Br^{82} space (lit.)
1.2
1.1
1.0
0.9
0.8
H$_2^3$O space (lit.)
2.4
2.3
2.2
2.1
2.0
1.9
intracellular vol. (lit.)
1.4
1.3
1.2
1.1
plasma Na$^+$ conc.
190
180
170
160
150
140
1.0
2.0
3.0
4.0
5.0
6.0
7.0
TIME (hrs.)

FIGURE 3. Changes in fluid compartment volumes and plasma Na^+ concentration in response to salt loading in NaCl fed duck, *Anas platyrhynchos*, which demonstrated nasal gland activity. Conditions of the salt load and coordinates of the measured responses are the same as those described in Figure 1.

□————□ Salt loaded duck C-2 #4.

○— — —○ Control salt water duck C-4 #5; no salt load.

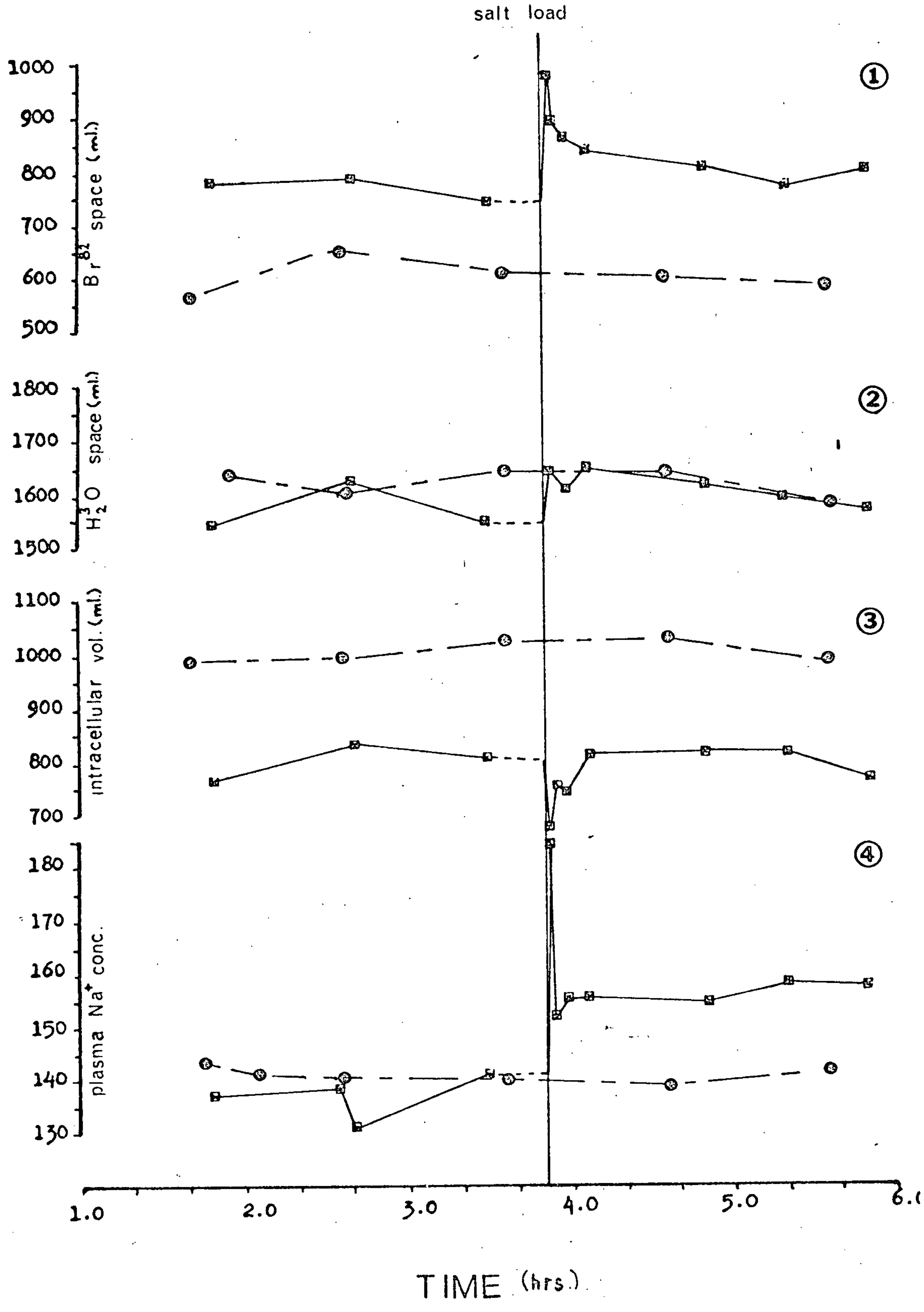

salt load
①
1000
900
800
700
600
500
Br^{82} space (ml.)
②
1800
1700
1600
1500
H_2^3O space (ml.)
③
1100
1000
900
800
700
intracellular vol. (ml.)
④
180
170
160
150
140
130
plasma Na^+ conc.
1.0
2.0
3.0
4.0
5.0
6.(
TIME (hrs.)

FIGURE 4. Changes in fluid compartment volumes and plasma Na^+ concentration in response to salt loading in a Glaucous-winged gull (Larus glaucescens) which demonstrated nasal gland activity. Conditions of the salt load and coordinates of the measured responses are the same as those described in Figure 1.

⊡————⊡ Salt loaded gull G-1.

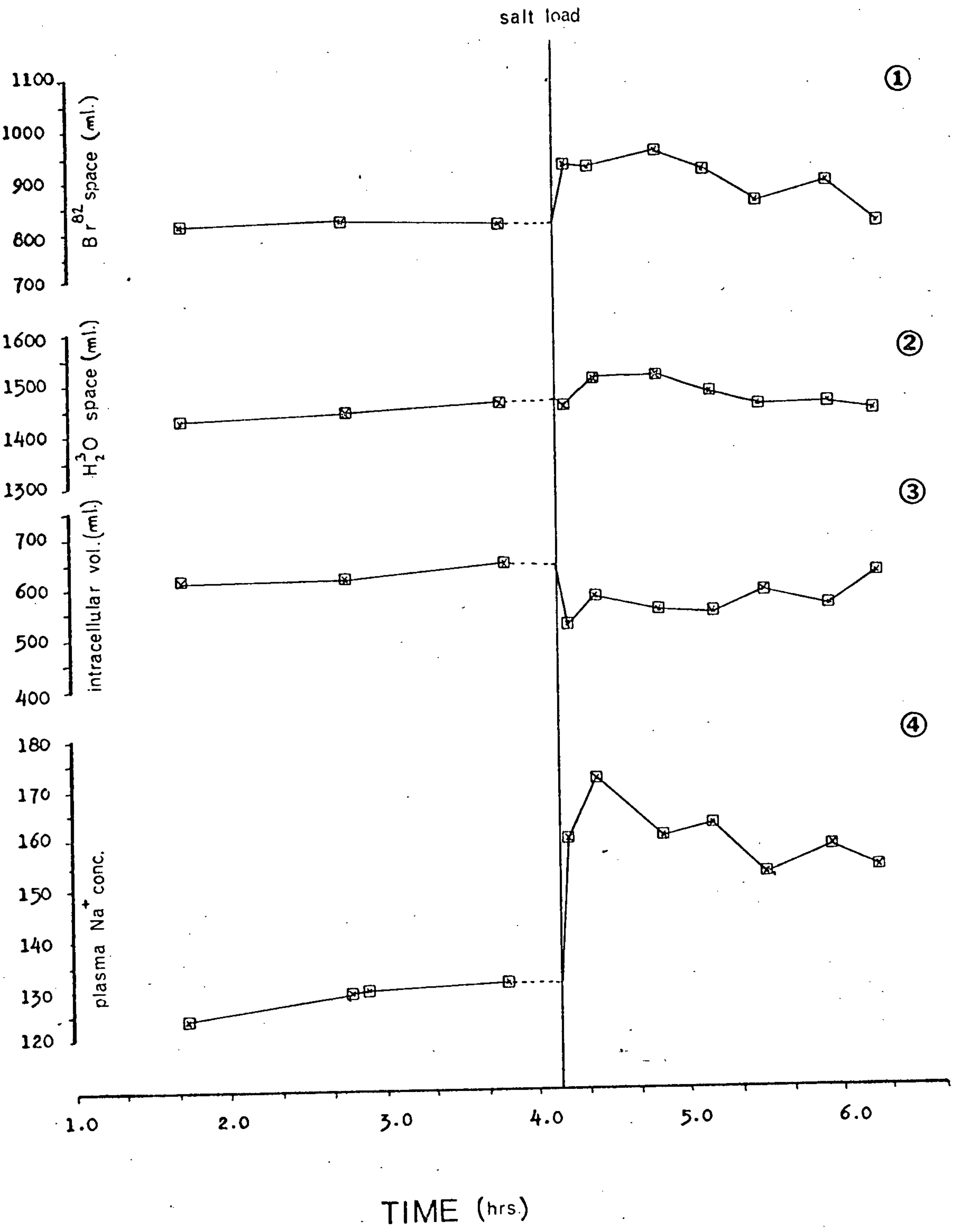

salt load
①
②
③
④
1100
1000
900
800
700
Br^{82} space (ml.)
1600
1500
1400
1300
H_2^3O space (ml.)
700
600
500
400
intracellular vol. (ml.)
180
170
160
150
140
130
120
plasma Na^+ conc.
1.0
2.0
3.0
4.0
5.0
6.0
TIME (hrs.)

FIGURE 5. Changes in fluid compartment volumes and plasma Na^+ concentration in response to salt loading in a White Leghorn rooster, *Gallus domesticus.* Conditions of the salt load and coordinates of the measured responses are the same as those described in Figure 1.

□——————□ Salt loaded rooster R-2; no nasal gland present.

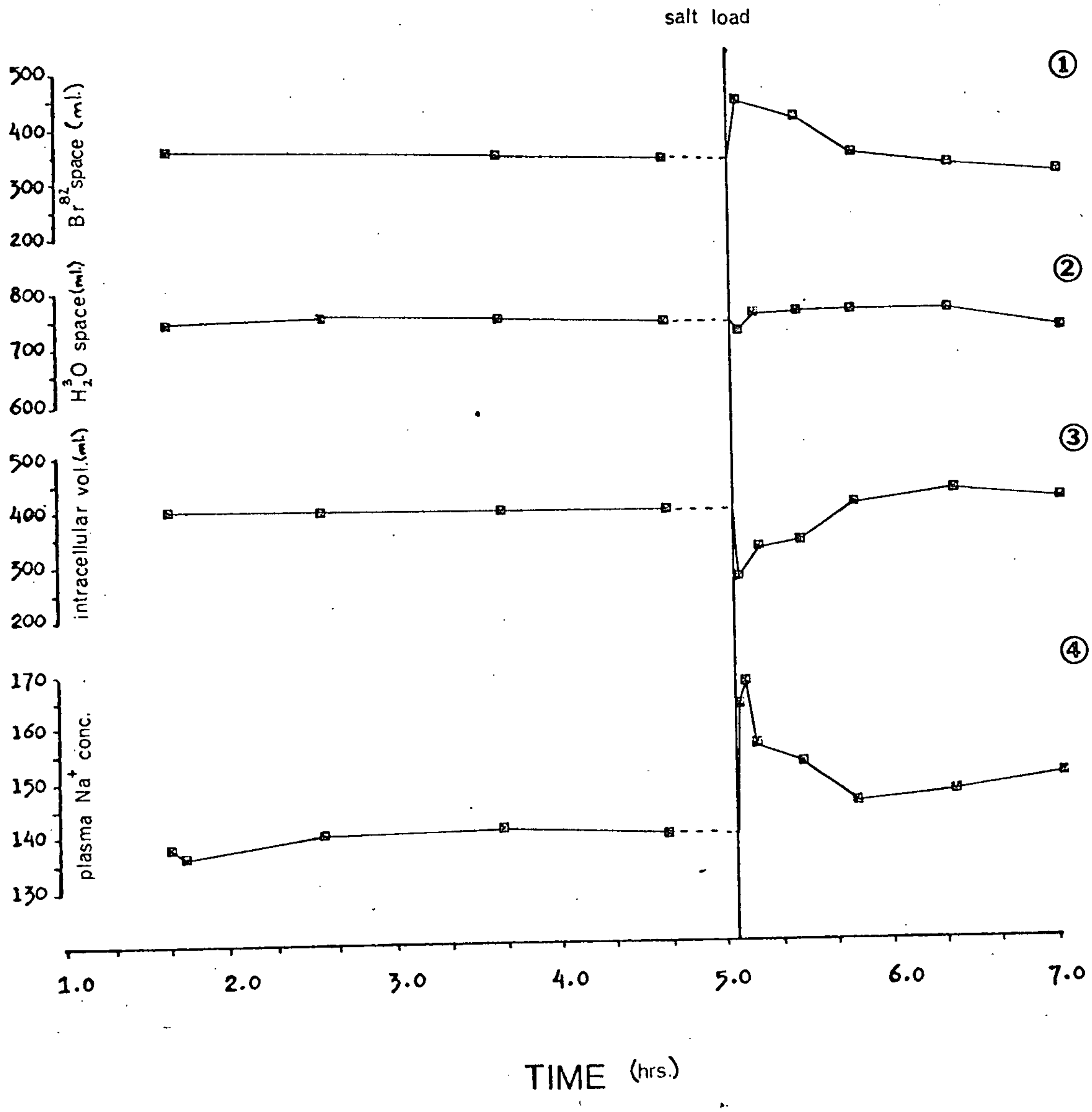
salt load
①
②
③
④
500
400
300
200
Br^{82} space (ml.)
800
700
600
H_2^3O space (ml.)
500
400
300
200
intracellular vol. (ml.)
170
160
150
140
130
plasma Na^+ conc.
1.0
2.0
3.0
4.0
5.0
6.0
7.0
TIME (hrs.)

FIGURE 6. Nasal gland responses as compared to changes in ECF volume after intravenous salt loading. Maximum post-salt load Br^{82} Space increases are plotted against volumes of collected nasal gland secretion. Fluid was collected until secretion stopped. ⊙ = NaCl solution (0.483N) fed ducks (*Anas platyrhynchos*); ● = fresh water fed ducks (*Anas platyrhynchos*); ◈ = gulls (*Larus glaucescens*).

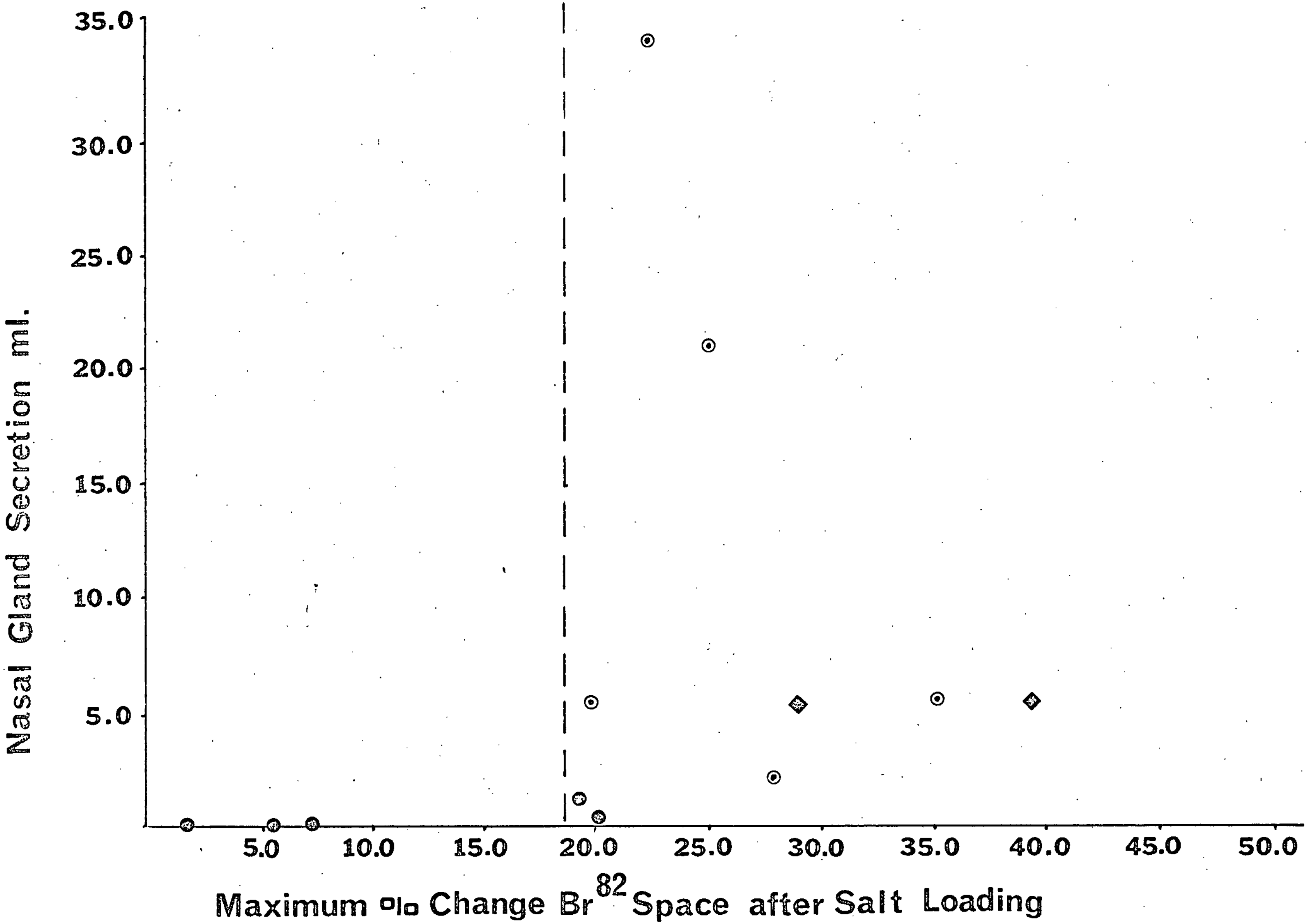
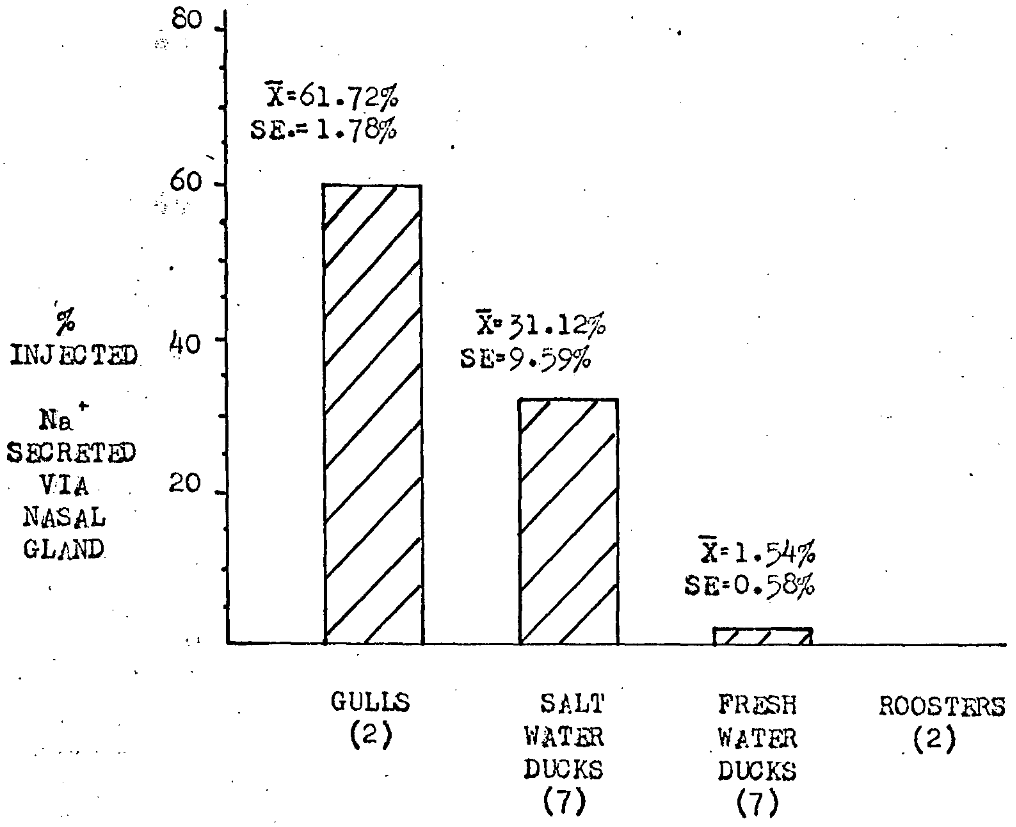
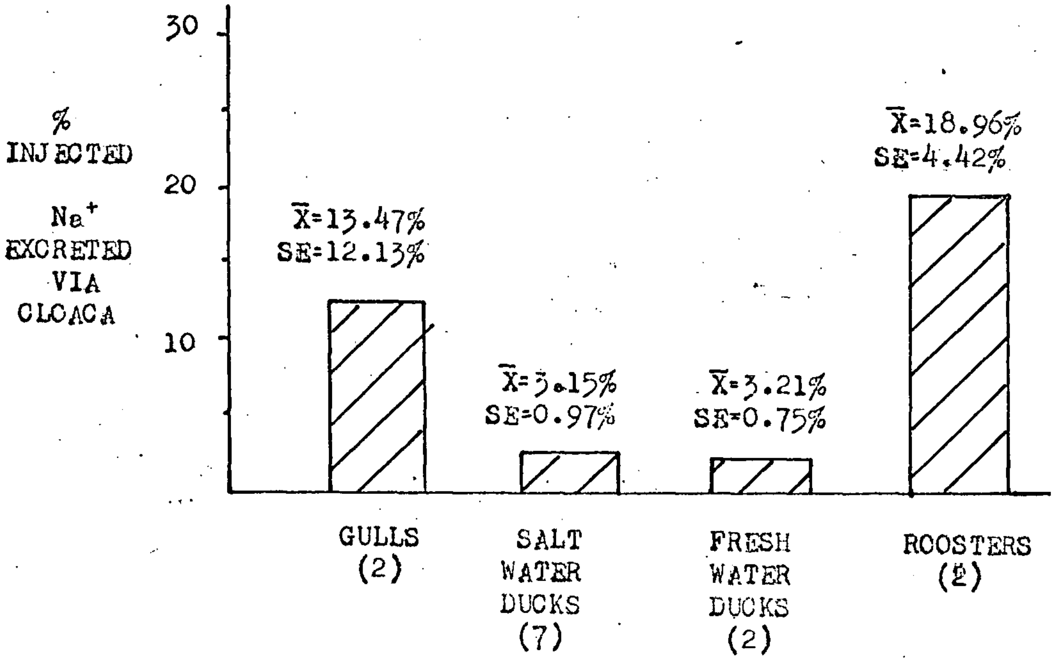

35.0
30.0
25.0
20.0
15.0
10.0
5.0
Nasal Gland Secretion ml.
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0
Maximum % Change Br82 Space after Salt Loading

FIGURE 7. Sodium elimination via cloacal and nasal gland routes in intravenously salt loaded gulls (Larus glaucescens), salt and fresh water fed ducks (Anas platyrhynchos), and White Leghorn roosters (Gallus domesticus). The amount of sodium is expressed as a percentage of the injected load (10 mEq/kg. body wt.). Means are presented with their standard errors. Sample sizes are indicated by the numbers in ( ).


80
60
40
20
%
INJECTED
Na$^+$
SECRETED
VIA
NASAL
GLAND
$\bar{X}$=61.72%
SE.=1.78%
$\bar{X}$=31.12%
SE=9.59%
$\bar{X}$=1.54%
SE=0.58%
GULLS
(2)
SALT
WATER
DUCKS
(7)
FRESH
WATER
DUCKS
(7)
ROOSTERS
(2)


30
20
10
%
INJECTED
Na$^+$
EXCRETED
VIA
CLOACA
$\bar{X}$=13.47%
SE=12.13%
$\bar{X}$=3.15%
SE=0.97%
$\bar{X}$=3.21%
SE=0.75%
$\bar{X}$=18.96%
SE=4.42%
GULLS
(2)
SALT
WATER
DUCKS
(7)
FRESH
WATER
DUCKS
(2)
ROOSTERS
(2)