METABOLISM OF INORGANIC CATIONS BY QUAIL (*COTURNIX COTURNIX*) DRINKING SOLUTIONS OF CaCl$_2$ AND MgCl$_2$

ERNEST J. WILLOUGHBY*

Department of Zoology, The University of Michigan, Ann Arbor, Michigan 48104

Abstract—1. In experiments to indicate the abilities of birds to tolerate and excrete Mg and Ca in drinking water, twenty-eight male quail received solutions of CaCl$_2$ or MgCl$_2$ perenterally for several days or in single doses. The concentrations of Ca, Mg, Na and K were subsequently measured in excretory fluids voided spontaneously or collected by intubating cloaca and rectum, and in plasma.

2. The physiological mechanisms of quail which deal with divalent cations are affected similarly by both Ca and Mg, without distinguishing sharply between the two.

3. Calcium appears to be less readily absorbed in the gut than Mg.

4. Excretion of Na is increased by intake of CaCl$_2$ or MgCl$_2$.

5. Water appears to be absorbed from ureteral urine in cloaca or rectum.

6. The effects of CaCl$_2$ and MgCl$_2$ on salt metabolism of the quail are complex.

INTRODUCTION

DURING the past two decades physiologists have investigated the abilities of various birds to drink hyperosmotic saline solutions and to obtain physiologically useful water from them. These studies have contributed to an understanding of the adaptive specializations of birds that live in a variety of habitats, including marine situations, salt marshes, and deserts, where the salinity or scarcity of water make it difficult to maintain homeostasis (for reviews see Bartholomew & Cade, 1963: Cade, 1964; Dawson & Bartholomew, 1968). These studies have dealt only with metabolism of NaCl and the tolerance of birds to various concentrations of NaCl or sea water (for example, see Bartholomew & Cade, 1963; Cade, 1964; Dawson *et al.*, 1965; MacMillen & Trost, 1966; Harriman, 1967; Harriman & Nance, 1968), and the results have been interpreted only in terms of the osmotic effects of NaCl. Since sea water contains appreciable concentrations of Ca and Mg—10·4 mM Ca, 54·7 mM Mg (Barnes, 1954)—and since saline desertic waters frequently contain considerable amounts of Ca and Mg (Margat, 1961; Williams & Siebert, 1963), it is desirable to know what physiological effects these biologically

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* Present address: Division of Science & Mathematics, McKendree College, Lebanon, Illinois 62254.

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active ions may have on birds. Such knowledge would contribute to a better understanding of the physiological adaptations of birds for utilizing such waters. Therefore, I did some experiments with quail (*Coturnix coturnix*) to learn something about their abilities to tolerate and excrete Mg and Ca in their drinking water, the excretory pathways involved in their salt metabolism, and the possible function of the cloaca and rectum in salt and water balance.

I chose the 120-g quail for the experiments because it can be kept in good condition in small individual cages that take up relatively little space, and it is big enough to allow sampling of blood and urine in volumes sufficient for analysis of several cations per sample. The quail employed came from a breeding colony maintained in the Department of Zoology, the University of Michigan, and were derived from the Japanese subspecies, *C. c. japonica*. The species occupies a wide geographic range in Eurasia and Africa.

MATERIALS AND METHODS

Twenty-eight male quail aged 6–22 months were employed. The mean body weight of these birds during experiments was 119·8 *g*, range 85·9–156·8 *g*, s.d. 14·1. The birds were housed in individual wire cages 30·4 cm (12 in.) to a side, and were maintained on a commercial diet (Purina Game Bird Breeder Layena) which I found to contain 0·230 m-equiv/g Mg, 1·05 m-equiv/g Ca, 0·141 m-equiv/g Na, and 0·305 m-equiv/g K.

Acute and chronic loading regimens

To study the effects of the intake of $CaCl_2$ and $MgCl_2$ on cation metabolism in the quail, I gave solutions of these salts to the birds either over a period of days, referred to as "chronic loading", or in a single dose, termed "acute loading". The regimen for chronic loading consisted of substituting solutions of $MgCl_2$ or $CaCl_2$ (or distilled water in controls) for their usual drinking water for 4 or more days. In some cases individual quail would not drink the higher concentrations of $CaCl_2$ or $MgCl_2$, and so these birds were given regular doses of the solution by stomach tube for at least 3 days prior to the next step. Food and fluid were then removed from the cages for 20–24 hr, and at the end of this period of fasting, 3 ml of the solution were injected into the crop or stomach through polyethylene tubing (PE-50/S36, Clay-Adams, Inc., New York) inserted through the esophagus, and the fluids voided by the birds over the subsequent 4 or 5 hr were collected. Acute loading consisted of removing food and water from cages of birds previously maintained on tap water, injecting 3 ml (in a few instances 5 ml, see Table 1) of the designated fluid by stomach tube 20–24 hr later, then collecting fluids voided during the subsequent 4–5 hr.

Methods of collecting urinary fluids

Two methods were employed in collecting the fluids voided by the experimental quail. In one series of experiments, the birds were placed in individual wire cages (their usual living quarters) in a darkened room over pans containing light mineral oil about 1 cm deep so that as the birds voided the fluid spontaneously, it fell into the oil and sank. These fluids were then aspirated from beneath the oil at the end of the collection period in a Pasteur capillary pipette and mixed so that no distinction was made between urinary fluid and any fluid that might have passed unabsorbed through the gut. The fluids thus collected will be referred to as "spontaneously voided urine". Fasting of the quail before beginning the collection of the fluids minimized fecal contamination.

The second method of collecting fluid was designed to obtain urine directly as it passed into the cloaca from the ureters. This would minimize both contamination by unabsorbed fluid from the gut and the effects of cloacal or rectal reabsorption of water or solutes in the

urine. A collecting device of polyethylene tubing (Clay-Adams, Inc.) and rubber balloons was fashioned (Fig. 1) so that when it was inserted into the cloaca and rectum of the quail and held in place by stitches between the polyethylene sheet collar and the skin surrounding the cloaca, one tube opened near the ureter on one side of the cloaca, and the other tube extended into the large intestine and opened near the level of the paired ceca. During the insertion of this device, birds were anaesthetized with 0·40 ml of Equi-Thesin (Jensen-Salisbery Laboratories, Kansas City, Mo.) injected into the pectoral muscles. The quail remained under the effects of the drug for varying periods during the subsequent injection of the salt solution by stomach tube and collection of fluids. The quail then were held in individual cages in a darkened room while the fluids accumulated in the balloons attached to the tubes. This method of fluid collection is subsequently referred to as "intubation".

In an effort to check against contamination of urine by intestinal fluids, the solutions injected into the crops of the intubated quail were colored by dye (Fast Green FCF), which passed unabsorbed through the gut. Rarely was fluid in the urinary collecting bag colored by dye, whereas about one-third of the collections in the intestinal bag had traces of green dye. Urine that was colored by green dye was discarded.
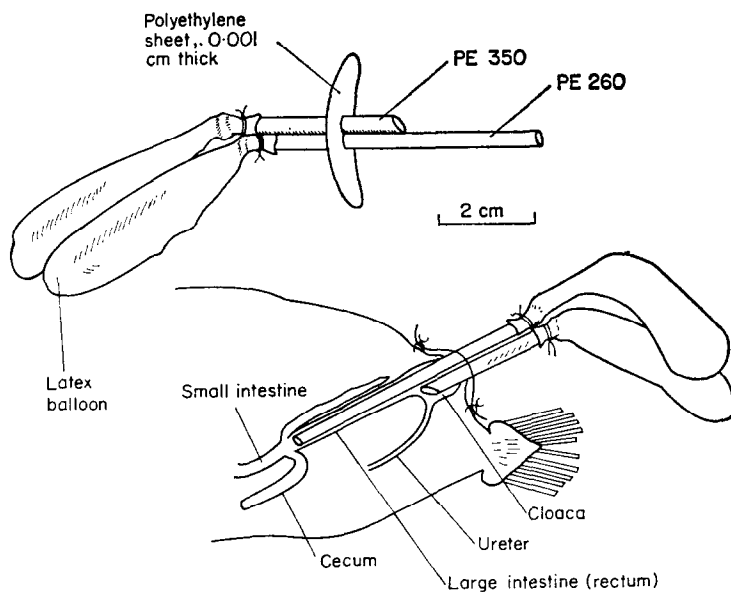

Fig. 1. Diagram of the device employed to collect ureteral urine from the cloaca, and intestinal fluids from the anterior end of the rectum, showing the manner of insertion. The diagram shows the quail on its back, as it was held during insertion of the tubes.

Blood samples

Blood was obtained from unanaesthetized quail by heart puncture with a 26 ga., 1 in. long hypodermic needle on a 1 cm³ heparinized glass tuberculin syringe. The needle was inserted between the furculum along the dorsal side of the sternum. Sodium-heparin was used as the anticoagulant, and distilled water drawn into heparinized syringes and treated in the same manner as the blood had 1·5–3·0 m-equiv/l. Na. Therefore, the plasma Na values presented here must be considered to be elevated by 2–3 per cent owing to the presence of

the heparin. Four-tenths to 0·8 ml of blood was drawn into the syringe, and the syringe was rocked gently to mix the blood with the heparin. The blood was then placed in capillary tubes (Kimax No. 34500, 100 mm long) which were closed and centrifuged for 20 min at 3000 rev/min. The hematocrit was determined, the tube broken just above the packed cells, and the plasma sealed in the remainder of the capillary tube and stored in a freezer for subsequent analysis.

Treatment of urinary fluids

The fluid voided by the birds was centrifuged in 15 ml graduated tubes and the volume of solids (including uric acid and occasional small amounts of fecal material) and of clear fluid estimated. Concentrated nitric acid was then added to the fluid in the proportion of 0·1 ml acid to 1 ml of clear liquid, and the mixture was shaken and allowed to stand at room temperature overnight. The purpose of this acidification was to put into solution Mg and Ca that may have been in the form of insoluble carbonates or urates, and to release Na or K which might be bound as insoluble urates (the urates thus being converted to the less soluble uric acid with release of the metal ions). The next day the acidified fluids were centrifuged again and the clear supernate sealed in capillary tubes and frozen for future analysis. In calculating the ion concentrations of the urinary fluids as they came from the quail, all subsequently determined values were multiplied by the appropriate factor owing to the dilution of the fluid with the acid, so that the concentrations given in the results reflect total ions excreted per volume of water, although some of these ions may have initially been in insoluble compounds.

Ion analysis

Sodium and potassium were determined by flame photometry (Coleman Model 21 Flame Photometer). Magnesium was determined with the dye Clayton Yellow as described by Natelson (1961, in conjunction with a Coleman Junior Spectrophotometer Model 6C.

Calcium was determined by two methods. For plasma, total Ca and Mg were determined by a modification of the method described by Grette (1953), and the concentration of Ca was taken as the difference between this value and the value for Mg alone as determined by the use of Clayton Yellow. For urinary fluids, Ca was determined by flame photometry using standards that contained the same ranges of Na, K and Mg as the samples. Precision of these methods was within 6 per cent or better.

RESULTS

Plasma ions and hematocrit

Table 1 lists plasma values of birds subjected to both chronic and acute loading. Birds given chronic loads of $MgCl_2$ had significantly elevated hematocrits ($P < 0.05$, two-tailed t-test) compared to the control group given distilled water, whereas none of the other experimental groups had hematocrits significantly different from the controls. Quail receiving chronic loading of $MgCl_2$ showed reduced levels of Ca in the plasma. In birds receiving 0·084 M $MgCl_2$ this resulted in a level of plasma Ca significantly different from the controls ($P < 0.02$, two-tailed t-test). Quail receiving loads of $MgCl_2$ showed a tendency to have elevated Mg concentrations in the plasma (0·099 M $MgCl_2$ acute, $P < 0.05$; 0·084 M $MgCl_2$ chronic, $P \ll 0.01$). Sodium concentrations in the plasma varied considerably, but showed no significant change relative to experimental regimens. Potassium showed no significant changes in relation to regimen.

TABLE 1—PLASMA ION CONCENTRATIONS AND HEMATOCRIT OF QUAIL DRINKING OR RECEIVING BY STOMACH TUBE SOLUTIONS OF $MgCl_2$ AND $CaCl_2$

Regimen*	Hematocrit $\bar{X} \pm 2$ S.E.$_{\bar{x}}$, (N)	Ca	Mg	Na	K
		m-equiv/l, $\bar{X} \pm 2$ S.E.$_{\bar{x}}$, (N)			
Dist. H_2O, acute	0·51 ± 0·030 (6)	4·57 ± 0·30 (6)	2·27 ± 0·26 (6)	160·7 ± 4·58 (6)	2·47 ± 0·47 (6)
Dist. H_2O, chronic	0·51 ± 0·024 (9)	4·18 ± 0·20 (9)	1·92 ± 0·16 (9)	157·3 ± 7·40 (9)	2·91 ± 0·45 (9)
0·068 M $MgCl_2$, chronic	0·58 ± 0·038 (6)	4·07 ± 0·29 (6)	1·92 ± 0·14 (6)	161·8 ± 9·05 (5)	2·64 ± 0·29 (5)
0·084 M $MgCl_2$, chronic	0·56 ± 0·036 (6)	3·73 ± 0·26 (6)	2·33 ± 0·18 (6)	165·0 ± 8·93 (5)	2·72 ± 0·71 (5)
0·099 M $MgCl_2$, acute	0·48 ± 0·017 (5)	4·14 ± 0·20 (5)	2·68 ± 0·22 (5)	157·0 ± 6·19 (5)	2·08 ± 0·27 (5)
0·072 M $CaCl_2$, chronic	0·52 ± 0·030 (8)	4·18 ± 0·17 (6)	1·83 ± 0·25 (6)	146·7 ± 6·86 (8)	2·87 ± 0·48 (8)
0·090 M $CaCl_2$, chronic	0·52 ± 0·015 (5)	3·98 ± 0·29 (5)	2·02 ± 0·16 (5)	142·5 ± 14·73 (4)	2·80 ± 0·74 (4)
0·107 M $CaCl_2$, acute	0·52 ± 0·031 (7)	4·00 ± 0·16 (7)	1·96 ± 0·18 (7)	156·4 ± 6·11 (5)	2·42 ± 0·54 (5)

* "Acute" means that birds received 5 ml of fluid 4–5 hr before sampling of blood, after being maintained on tap water. "Chronic" regimen as described in text.

Cations in spontaneously voided urinary fluids

Table 2 lists ion concentrations in the fluid voided spontaneously by birds subjected to chronic loading regimens. Some individuals receiving $MgCl_2$ voided fluid having concentrations of Mg in excess of that in the fluids they consumed. The quail receiving $MgCl_2$ solutions produced fluid with very significantly elevated concentrations of Ca, and the converse is true for the quail receiving $CaCl_2$ solutions.

All the quail that received loads of Ca and Mg produced excretory fluids with elevated concentrations of Na. There was no significant change in concentrations of K.

Cations in urine collected by intubation of the cloaca

Table 3 lists ion concentrations in urine taken from the cloaca at the opening of the ureter in quail subjected to acute and chronic loading regimens. Calcium did not vary significantly in relation to regimen, unlike the situation noted in the spontaneously voided fluids. Levels of Mg were significantly elevated in urine of quail subjected to Mg loading, but not in that of birds subjected to Ca loading. With Mg loading the concentration of Mg in urine collected by intubation was much lower than in the spontaneously voided fluid (compare with Table 2). Concentrations of Na did not vary significantly with regimen. Concentrations of K also appeared not to vary significantly, except possibly in the group receiving chronic loading with 0.072 M $CaCl_2$, where urinary K was considerably lower than in any of the other groups. However, it is clear that a larger sample size is needed to confirm whether or not this apparent decline is significant.

It is noteworthy that urine from intubated quail invariably had higher concentrations of K than did spontaneously voided fluids.

Rates of ion excretion

Table 4 lists average rates of ion excretion during the 4–5 hr period of fluid collection in all experimental groups. The values from intubated quail represent the sum of values from the intestinal and cloacal samples. The intubated quail had significantly elevated rates of water excretion compared to the birds that were voiding spontaneously.

In most instances, rates of ion excretion reflected the changes in concentrations noted above. Thus, birds receiving chronic loads of Mg and Ca had significantly elevated rates of excretion of Na. Intubated quail had highly significantly elevated rates of K loss compared to the other quail, owing both to their increased urinary concentration of K and their increased rate of fluid production. Loading with Mg elevated rates of Ca excretion by birds voiding spontaneously, and the converse was true for birds given Ca loads. However, intubated quail did not show significant increases in excretion of Ca when given Mg, and although their rates of Ca excretion were somewhat elevated when they were given Ca, these rates were far below those for comparable birds that were voiding spontaneously.

TABLE 2—CONCENTRATION OF CATIONS IN SPONTANEOUSLY VOIDED URINARY FLUID OF QUAIL SUBJECTED TO CHRONIC LOADING REGIMENS

Regimen	Volume of fluid voided ml, $\bar{X} \pm 2$ S.E.$_{\bar{x}}$ (N), range	m-equiv/l., $\bar{X} \pm 2$ S.E.$_{\bar{x}}$ (N), range			
		Ca	Mg	Na	K
Dist. H₂O	1·49 ± 0·52 (9), 0·75–2·20	8·7 ± 3·9 (9), 2·6–16·6	4·8 ± 2·0 (9), 1·3–9·6	9·3 ± 3·6 (9), 3·8–17·6	35·2 ± 12·5 (9), 21–73
0·068 M MgCl₂ (136 m-equiv/l. Mg)	1·01 ± 0·44 (6), 0·40–1·75	21·3 ± 10·3 (6), 0–35	121·5 ± 30·4 (6) 56–158	41·5 ± 14·9 (6), 18–64·8	17·0 ± 4·9 (6), 9–24
0·084 M MgCl₂ (168 m-equiv/l. Mg)	1·19 ± 0·75 (5), 0·2–2·0	52·0 ± 34·4 (5), 11–93	128·0 ± 53·9 (5), 46–210	39·6 ± 14·7 (5), 25–65	24·6 ± 8·3 (5), 15–36
0·072 M CaCl₂ (144 m-equiv/l. Ca)	1·27 ± 0·37 (8), 0·6–1·8	125·4 ± 61·7 (7), 17–247	15·2 ± 6·8 (7), 3·1–26·2	36·7 ± 37·0 (8), 4·8–163	41·5 ± 13·9 (8), 17–76
0·090 M CaCl₂ (180 m-equiv/l. Ca)	1·58 ± 0·73 (5), 0·35–2·40	187·6 ± 50·0 (5), 141–271	20·3 ± 5·7 (5), 12·2–28·2	37·1 ± 17·7 (5), 23·1–70·7	44·9 ± 10·0 (5), 34–58

TABLE 3—CONCENTRATION OF CATIONS IN URINE COLLECTED FROM THE CLOACA AT THE MOUTH OF A URETER IN QUAIL SUBJECTED TO ACUTE AND CHRONIC LOADING REGIMENS

Regimen	Ca	Mg	Na	K
	m-equiv/l., $\bar{X} \pm 2$ S.E.$_{\bar{x}}$, (N), range			
Dist. H_2O, acute	$6 \cdot 1 \pm 2 \cdot 9$ (14), $1 \cdot 1$–$18 \cdot 9$	$18 \cdot 9 \pm 5 \cdot 7$ (14), $6 \cdot 2$–$37 \cdot 9$	$14 \cdot 4 \pm 5 \cdot 9$ (14), $2 \cdot 7$–$39 \cdot 8$	$91 \cdot 5 \pm 16 \cdot 0$ (14), 36–144
$0 \cdot 068$ M $MgCl_2$, acute (136 m-equiv/l. Mg)	$4 \cdot 7 \pm 5 \cdot 9$ (8), $0 \cdot 2$–$25 \cdot 0$	$60 \cdot 1 \pm 12 \cdot 9$ (8), $32 \cdot 4$–$84 \cdot 2$	$10 \cdot 1 \pm 6 \cdot 1$ (8), $2 \cdot 7$–$28 \cdot 6$	$88 \cdot 1 \pm 15 \cdot 2$ (8), 58–117
$0 \cdot 068$ M $MgCl_2$, chronic	$5 \cdot 9 \pm 9 \cdot 5$ (5), $0 \cdot 6$–$24 \cdot 8$	$46 \cdot 3 \pm 19 \cdot 9$ (5), $26 \cdot 4$–$83 \cdot 5$	$18 \cdot 0 \pm 15 \cdot 5$ (5), $3 \cdot 9$–$47 \cdot 0$	$125 \cdot 0 \pm 123 \cdot 5$ (5), 42–371
$0 \cdot 072$ M $CaCl_2$, acute (144 m-equiv/l. Ca)	$8 \cdot 9 \pm 5 \cdot 3$ (8), $0 \cdot 9$–$22 \cdot 9$	$24 \cdot 5 \pm 2 \cdot 7$ (8), $19 \cdot 9$–$29 \cdot 6$	$12 \cdot 4 \pm 5 \cdot 7$ (8), $2 \cdot 7$–$26 \cdot 9$	$82 \cdot 0 \pm 16 \cdot 7$ (8), 46–117
$0 \cdot 072$ M $CaCl_2$, chronic	$3 \cdot 4 \pm 1 \cdot 5$ (5), $0 \cdot 9$–$5 \cdot 0$	$17 \cdot 1 \pm 3 \cdot 9$ (5), $12 \cdot 1$–$23 \cdot 3$	$9 \cdot 0 \pm 3 \cdot 8$ (5), $4 \cdot 4$–$14 \cdot 3$	$49 \cdot 2 \pm 15 \cdot 7$ (5), 29–67

Intubated quail received additional Mg when they were anaesthetized (0·4 ml of Equi-Thesin contains 0·14 m-equiv. Mg), which may account for the elevated rate of excretion of Mg in the intubated controls receiving distilled water as compared to controls voiding spontaneously.

DISCUSSION

Plasma ions and hematocrit

The elevation of the hematocrit of quail subjected to chronic Mg loading may indicate a loss of water from the plasma. It is not known whether this apparent water loss is the result of a shift of water and electrolytes to a different fluid compartment, or whether it represents a dehydration of the organism. The regimens of $CaCl_2$ did not have this effect, although the $CaCl_2$ solutions given to the quail had the same osmosity as did the $MgCl_2$ solutions; so the difference cannot result from differences in osmotic concentration of the solutions. If dehydration did occur, it may be the result of reduced absorption of water in the gut owing to a purgative action by $MgCl_2$. Cunningham (1933) noted that high levels of Mg in the food of rats gave them diarrhea.

The reduced concentration of Ca in plasma of quail subjected to chronic Mg loading indicates a slight loss of Ca from the plasma. It is intriguing that the sum of plasma Mg and plasma Ca in all chronically loaded birds remained quite constant—near 6·0 m-equiv/l.—regardless of concentration of $MgCl_2$, which suggests a reciprocal relationship between levels of Ca and Mg in plasma of birds subjected to chronic Mg loading. Similar relationships have been observed in

TABLE 4—RATES OF EXCRETION OF CATIONS BY QUAIL DURING THE 4 TO 5 HR AFTER INJECTION BY STOMACH TUBE OF 3 ML OF THE RESPECTIVE FLUIDS

Regimen and method of collecting fluid*	Volume of fluid voided $\bar{X}\pm 2$ S.E.$_{\bar{x}}$ (N), range	Ca	Mg	Na	K
		m-equiv/hr $\times 10^3$, $\bar{X}2$ S.E.$_{\bar{x}}$ (N), range†			
Dist. H_2O, spontaneously voiding, chronic	0·323±0·113, (9), 0·165–0·689	2·45±0·98, (9), 0·63–4·26	1·39±0·51, (9), 0·29–2·55	3·12±1·76, (9), 0·8–9·1	10·57±4·13, (9), 4·93–23·15
Dist. H_2O, intubation, acute	0·484±0·115, (14), 0·089–0·913	1·93±0·92, (14), 0·23–5·49	7·02±1·69, (14), 1·09–12·96	3·45±1·15, (13), 1·22–8·36	33·10±12·62, (13), 6·54–91·3
0·068 M $MgCl_2$, spontaneously voiding, chronic	0·224±0·100, (6), 0·088–0·392	5·26±4·01, (6), 0·0–12·7	24·18±7·78, (6), 13·5–40·9	7·53±1·09, (6), 5·72–9·41	3·62±1·93, (6), 1·66–8·23
0·068 M $MgCl_2$, intubation, chronic	0·524±0·175, (6), 0·148–0·686	1·55±0·83, (6), 0·71–2·74	22·24±8·28, (6), 11·18–37·50	5·07±3·13, (6), 2·24–11·68	32·88±13·53, (6), 6·29–56·47
0·068 M $MgCl_2$, intubation, acute	0·529±0·122, (10), 0·206–0·881	3·10±4·01, (10), 0·0–20·73	25·58±5·87, (10), 12·30–42·25	4·04±2·12, (10), 0·56–11·96	36·54±9·85, (10), 21·41–65·45
0·084 M $MgCl_2$, spontaneously voiding, chronic	0·262±0·165, (5), 0·044–0·443	11·96±9·17, (5), 1·10–27·00	32·32±22·80, (5), 4·56–65·60	9·70±6·28, (5), 1·28–20·40	6·52±4·61, (5), 0·66–14·32
0·072 M $CaCl_2$, spontaneously voiding, chronic	0·273±0·078, (8), 0·133–0·396	34·98±19·05, (7), 2·77–86·70	4·44±2·40, (7), 0·51–10·04	9·45±7·88, (8), 0·78–33·90	10·63±3·36, (8), 2·77–16·60
0·072 M $CaCl_2$, intubation, chronic	0·733±0·173, (5), 0·521–0·928	3·86±1·26, (5), 2·32–5·66	13·21±4·27, (5), 6·29–18·43	7·45±1·82, (5), 5·01–10·53	39·41±12·55, (5), 24·00–55·50
0·072 M $CaCl_2$, intubation, acute	0·462±0·100, (8), 0·270–0·689	4·35±1·59, (8), 1·77–8·79	10·64±1·95, (8), 6·00–14·14	4·39±1·68, (8), 1·05–9·12	36·87±13·39, (8), 23·92–81·55
0·090 M $CaCl_2$, spontaneously voiding, chronic	0·327±0·156, (5), 0·076–0·519	56·08±24·40, (5), 20·6–80·9	6·53±4·29, (5), 1·91–14·60	9·60±2·68, (5), 5·4–13·1	13·26±4·79, (5), 4·2–17·7

* See text for details.

† To obtain actual values, multiply all figures expressed as m-equiv/hr by 10^{-3}.

laboratory rats. Cunningham (1933) observed that rats fed diets containing supplementary Mg had elevated serum Mg and slightly lowered serum Ca. Alcock & MacIntyre (1962) observed that rats fed a diet deficient in Mg had increased plasma Ca and decreased plasma Mg; and Clark (1968) observed a slight lowering of serum Ca in rats that were given solutions of $MgCl_2$ to drink.

Cations in urinary fluid

The increased rates of excretion of Ca and Mg by quail receiving loads of Mg and Ca, respectively, agree with observations on mammals. Thus Malcolm (1905) observed that when dogs were given additional $MgCl_2$ in their food, there was an increase in urinary output of Ca, although additional $CaCl_2$ in the food did not appear to elevate urinary Mg. Mendel & Benedict (1909a) injected $MgSO_4$ or $MgCl_2$ subcutaneously or intraperitoneally into dogs, cats and rabbits, and observed increases in urinary output of both Ca and Mg. When they injected $CaCl_2$ intravenously into rabbits and dogs, Mendel & Benedict (1909b) observed increases in urinary Ca and Mg. More recently, Samiy *et al.* (1960) found that when dogs received infusions of $MgCl_2$, the excretion of Ca was markedly increased, and when they were infused with $CaCl_2$ the excretion of Mg was slightly increased. Clark (1968) found that rats drinking solutions of $MgCl_2$ had significantly increased urinary Ca, while Alcock & MacIntyre (1962) observed that rats eating a diet deficient in Mg had decreased urinary excretion of Mg and Ca.

Explanations of this interaction of Mg and Ca have varied with experimental treatment. Results of studies involving dietary supplements of Mg and Ca tend to indicate that the presence of Mg in the gut enhances absorption of Ca (Clark, 1968). On the other hand, studies of absorption and excretion of Ca and Mg by rats fed diets deficient in one or the other of these elements have been interpreted to indicate that the deficiency of one of the ions enhances intestinal absorption of the other (Alcock & MacIntyre, 1962). Therefore, it appears unlikely that the effect is owing only to the activity of a common transport mechanism in the intestine. Studies of the action of the parathyroid gland on levels of plasma Ca in laboratory rats injected with $MgCl_2$ indicate that hypermagnesemia inhibits parathyroid activity (Gitelman *et al.*, 1968), suggesting that the parathyroids may mediate the interaction between Ca and Mg. Studies of the renal excretion of Mg and Ca of dogs infused with $MgCl_2$ and $CaCl_2$ suggest also that Mg and Ca compete for a common renal tubular reabsorptive mechanism (Samiy *et al.*, 1960). In any case, it appears that the regulatory systems involved are not capable of clearly differentiating the two divalent cations in these mammals and in the quail.

While my observations on excretion of Ca and Mg in quail are consistent with findings for various mammals, and may reflect activities of homologous regulatory systems, it should be noted that the avian urinary tract empties into the cloaca where the urine may be modified by mixing with intestinal fluids and feces, or by reabsorptive and secretory activities of the cloaca or large intestine. Urine characteristically moves into the large intestine right up to the ceca after it enters the

cloaca from the ureters (Skadhauge & Schmidt-Nielsen, 1965; Skadhauge, 1967, 1968; Nechay et al., 1968). Therefore, I performed the experiments with the intubated quail in an attempt to see what effect the cloaca and intestine may have on excretion of cations and water. Taken at face value my results indicate that about half of the water in the urine is reabsorbed in the cloaca or rectum. This is consistent with observations by Skadhauge (1968) on roosters that had been deprived of water for 36 hr. My results also show that the Ca in ureteral urine did not reflect the Ca loads administered. Much of the Ca therefore may pass through the gut without being absorbed, or it may be secreted back into the intestinal or cloacal lumen after being absorbed. I believe it is most likely that not all of the Ca was being absorbed in the gut, but was precipitated as carbonate in the intestine and then mixed with the urine in the rectum and cloaca before the urine was voided. It appears, though, that the Mg was absorbed in the intestine, and excreted through the kidney. This difference between absorption of Ca and of Mg in the intestine would account for the greater effect of Mg on the birds' systems and the consequent enhancement of Ca excretion by birds receiving loads of Mg.

There is a possibility that the additional handling of the quail attendant to the insertion of the collecting device may have induced diuresis (Hester et al., 1940), which could partly account for the higher rates of water loss in intubated birds. The anaesthetic contained 177 mM/l. $MgSO_4$, and the additional Mg thus administered may account for increased excretion of Mg in intubated birds compared to their spontaneously voiding counterparts. The anaesthesia and intubation may have been factors that contributed to the three to sixfold higher rates of K excretion by these birds than in normal birds, although the possibility that K is normally reabsorbed in the rectum or cloaca cannot be eliminated. In this regard it is well to cite the in vivo perfusion studies on chicken cloaca by Skadhauge (1967) which demonstrated a net secretion of K into the lumen of the coprodeum and large intestine when the concentration of K in the perfusate was less than 90 m-equiv/l. It also appears relevant that the rate of excretion of K by roosters which had been deprived of water for about 36 hr did not differ significantly between birds voiding urine spontaneously and birds in which urine was collected through polyethylene funnels sewn over the ureteral openings (Skadhauge, 1968). Thus it appears that quail and chicken might differ in regard to the roles of the kidneys and of the cloaca and rectum in the excretion of K.

The increased excretion of Na by quail receiving loads of Ca and Mg is intriguing. The quail voiding urine spontaneously had concentrations of Na increased 4- to 5-fold when given $MgCl_2$ or $CaCl_2$, and their total rates of excretion of Na were 2 to 3 times higher than controls given distilled water. However, intubated birds did not show such clear-cut increases in rates of Na excretion when given Ca and Mg, and the concentration of Na in the ureteral urine of these birds did not vary significantly. This seems to indicate that Na was secreted into the cloaca or rectum of quail subjected to Mg and Ca loading, rather than excreted through the kidneys.

Spontaneously voided urine of ducks (*Anas platyrhinchos*) maintained on hypertonic saline had significantly higher concentrations of Ca than that of ducks maintained on tap water (Holmes *et al.*, 1968). It is also interesting in this connection that the clearances of Na and unbound Ca by dogs are linearly related under varying conditions of diuresis, salt loading, and glomerular filtration rate (Walser, 1961; Massry *et al.*, 1967). It is tempting to hypothesize that the simultaneous increases in urinary Na and Ca in the quail and duck are manifestations of the same physiological process. At any rate, it appears that the interactions between K, Na, Ca and Mg in the processes of avian salt metabolism are rather complicated; and investigators of utilization of sea water by birds should consider the complex physiological interactions of the various ions when interpreting their results.

Further studies are needed to define precisely the roles of intestinal absorption, renal excretion, and cloacal or rectal reabsorption and secretion (and nasal salt excretion in birds having functional nasal glands) in avian salt metabolism.

SUMMARY

To investigate how waters, such as saline desertic waters, that contain Mg or Ca as well as Na may affect the salt and water metabolism of birds that drink them, solutions of $CaCl_2$ and $MgCl_2$ were administered to quail (*Coturnix coturnix*) perenterally and then concentrations of cations in their plasma and urine were measured. Two methods of administering Ca and Mg to the quail were employed, namely (i) "acute loading" whereby quail were maintained on tap water, held 20–24 hr without food or water, then given 3–5 ml of $MgCl_2$ or $CaCl_2$ solution by stomach tube; and (ii) "chronic loading" whereby the quail were maintained on the designated salt solution for at least 4 days, and then were fasted and injected with the solution. Urinary fluids were collected during the 4–5 hr after injection of the salt solution by stomach tube, either as spontaneously voided urine, or by means of polyethylene tubes inserted into the cloaca and the rectum so as to collect ureteral urine that was not modified by contact with rectal fluids and mucosa.

Quail receiving chronic loading with 0·084 M $MgCl_2$ had significantly reduced plasma Ca; and these quail as well as those given acute loads of 0·099 M $MgCl_2$ had significantly elevated plasma Mg (plasma sampled 4–5 hr after injection of $MgCl_2$ by stomach tube).

The spontaneously voiding quail given loads of $MgCl_2$ had urine with significantly elevated concentrations of Ca, and the converse was true for quail given loads of $CaCl_2$. They also had elevated urinary concentrations of Na when given $MgCl_2$ or $CaCl_2$, but concentrations of K were not affected.

Intubated quail produced urine with increased concentrations of Mg when they received $MgCl_2$; but urinary concentrations of Ca and Na were not affected by Ca and Mg loading. The concentration of K in this urine was invariably higher than in spontaneously voided urine. Rates of fluid excretion of intubated quail were about twice as high as of spontaneously voiding quail.

The results indicate that (i) the regulatory system that deals with divalent cations in the quail is affected similarly by both Ca and Mg, and does not distinguish completely between the divalent cations; (ii) calcium appears to be less readily absorbed in the gut than is Mg; (iii) excretion of Na is increased by the intake of $CaCl_2$ or $MgCl_2$; (iv) water appears to be reabsorbed from ureteral urine in the cloaca or rectum; (v) excretion of K was unaffected by increased intake of $CaCl_2$ or $MgCl_2$; (vi) the effects of $CaCl_2$ and $MgCl_2$ on salt metabolism of quail are complex.

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