THE EFFECT OF ADRENOCORTICAL STEROIDS, SUBTOTAL AND TOTAL ADRENALECTOMY ON THE RENAL AND EXTRA-RENAL RESPONSES OF THE DOMESTIC DUCK (ANAS PLATYRHYNCHUS) TO HYPERTONIC SALINE LOADING

by

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ABSTRACT

The purpose of this investigation was to determine whether or not the adrenocortical steroids influence the activity of the avian supra-orbital gland.

In the intact saline loaded duck (Anas platyrhynchus), a diphasic excretory pattern was apparent, as an urinary diuresis was followed by activation of the supra-orbital glands. Variations in the circulating levels of adrenocortical steroids resulted in qualitative and quantitative changes in both phases of this response.

Injection of exogenous, cortisol, cortexone and aldosterone caused an initial increase in the rate of nasal discharge and a consequent increase in the total output of Na^+ and K^+. Conditions of selective adrenocortical activity, i.e., subtotal adrenalectomy, severely impaired the extra-renal response while total adrenalectomy completely obliterated the response to an osmotic stimulus. The activity of the supra-orbital gland in the totally adrenalectomized duck was restored after treatment with cortisol.

The renal phase in ducks treated with adrenocortical steroids was modified when compared to the control response. Administration of cortisol prior to saline loading resulted in a significantly lower total output of sodium although the volume of urine excreted did not differ from the control value. Treatment with aldosterone resulted in a significantly lower renal output of both Na^+ and K^+ as well as a decrease in urine volume. Cortexone and ACTH showed the control renal pattern of excretion.

Unilateral adrenalectomy caused no apparent change in the renal excretory pattern whilst total adrenalectomy caused a significant increase in the total volume of urine excreted as well as on increase in the total output of Na^+ and K^+. Totally adrenalectomized birds maintained on cortisol reverted to a normal renal excretory pattern.

The influence of adrenocortical steroids on the control of the avian supra-orbital gland is discussed.

It can be said with certainty that adrenocortical steroids influence the activity of the supra-orbital gland of the Domestic Duck (Anas platyrhynchus). Furthermore, cortisol administered to the adrenalectomized animal reactivates the gland suggesting that a glucocorticoid is essential for its function.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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Introduction

The chemical reactions associated with the metabolic processes in higher vertebrates appear to have become very specialized with respect to the pH optima and the tissue electrolyte composition. Consequently the vertebrates have evolved efficient excretory systems to maintain the constant milieu interieur of the animal against an ever changing external environment.

Thus fresh water vertebrates which are hypertonic to their environment continually lose tissue electrolytes and gain tissue water by the process of osmosis whereas in marine species where the tissues are hypotonic with respect to the environment, the opposite tendency occurs. In order to sustain the water and electrolyte composition of the tissues against the concentration gradient of the environment, the vertebrates have developed a specialized excretory system consisting of an ultra-filtration process whereby the aqueous solutes of the blood are filtered and selectively reabsorbed as the filtrate passes down a tubule (1). The vertebrate kidney is such a system.

With the possible exception of the amphibia certain members of all classes of vertebrates have developed the habit of periodically migrating from a fresh water to a marine environment and vice versa or occasionally adopting extremely arrid habitats. In order to withstand the environmental changes contingent upon such behavioural patterns, these animals have developed numerous adaptive mechanisms, one of which is an extra-renal excretory system.

The elasmobranch fishes, for instance, maintain high blood levels of urea so that their tissue fluids are hypertonic to a marine environment thus preventing dehydration (2). Fresh water teleosts eliminate water via the kidney and actively absorb sodium and chloride across the gill epithelium in order to maintain salt-electrolyte balance. In a marine environment however, which is hypertonic to its' body fluids, the teleost fish must conserve water. Smith has observed that in

order to cope with continuous dehydration certain marine fishes, unlike their fresh water counterparts, swallow large amounts of sea water and that both salts and water are taken up in the gut (3). The physiological problem then arises as to how the salts can be efficiently removed without a commensurate loss of water.

Recent work by Holmes (4) as well as Keys, Schlieper and others (5, 6, 7) has shown that the gill epithelium is the extra-renal site for the excretion of excess electrolytes. In other words a marine fish drinks sea water to compensate for water loss through dehydration but at the same time incurrs a salt load that cannot be excreted by the kidney without greater than equivalent losses of water. Thus the gill epithelium becomes the major organ of osmoregulation and an alternate pathway for the efficient removal of sodium and chloride.

The amphibians, a fresh water group of animals, have been unable to solve the physiological problems of adaptation to a marine life. Despite this fact, they have developed an extra-renal site for the active absorption of electrolytes and water. This site is the skin, the permeability of which is under the control of the amphibian posterior pituitary hormone, arginine vasotocin (8).

For terrestrial reptiles the problem of water balance is much the same as that of the marine teleost except that an inward salt gradient does not exist. Reptiles have developed a horny integument to prevent water loss by surface evaporation while water loss by a poorly vascularized kidney is small. The urine is greatly hypertonic to the blood, in fact in the snakes and lizards the urine is either solid or semi-solid (9). Marine reptiles on the other hand, have developed extra-renal sites for salt loss in order to cope with salts that might be ingested. In the sea turtle for example, a salt gland similar in structure to that of the marine bird elaborates a clear fluid concentrated in sodium chloride. This gland is situated behind the eye and is believed to be of different embryological origin than that of the marine bird (10). Schmidt-Nielsen has also

observed a salt gland in the Galapagos marine iguana (Amblyrhynchus cristatus) the only true marine lizard. Anatomical studies of the other marine reptiles, the sea snakes and the marine crocodiles have established the presence of large glands that may have a function similar to that of the avian salt gland (10).

Marine birds have developed a highly efficient extra-renal site for the excretion of salts. In fact, it has been demonstrated that the cormorant, Herring gull, Humboldt penguin, Brown pelican, Albatross, Eider duck and the Domestic duck are all capable of maintaining salt balance through an extra-renal excretory process. The site of the extra-renal response in marine birds is the paired supra-orbital glands which discharge a fluid via two collecting ducts into the anterior nasal cavity (11). This fluid is highly concentrated and contains large amounts of sodium and chloride with traces of potassium. The excretion of such a fluid is a process whereby marine birds can conserve water or conversely excrete heavy salt loads when exposed to a sea water diet for long periods of time.

Marine mammals maintain salt-water balance through the kidney which is evidently the only major site of electrolyte transfer. In any case, there appears to be no need for an extra-renal system because they feed on forms that are regulators. The seal, for example, feeds on fish which are nearly isotonic to its blood. However, whales and walruses whose diets may be predominantly squid, plankton and shellfish, invertebrates which are isotonic with respect to sea water, must eliminate large quantities of salt by a highly efficient kidney in order to prevent tissue dehydration (9). Terrestrial mammals rely wholly on the kidney for water loss or retention and selective electrolyte transfer even though certain modifications do exist in animals such as the Kangaroo rat. This animal can survive on a dry diet while excreting urine as concentrated as 900 mM/liter with respect to chloride compared to a maximum concentration of 370 mM/liter in man (9).

It has been shown that oral and parental administration of sodium chloride

to certain marine birds stimulates an extra-renal excretion of electrolytes via the supra-orbital gland. This response is probably of an osmoregulatory nature since intra-venous administration of sucrose also triggers the mechanisms associated with the activation of this extra-renal response. Even though we suspected that the salt gland was under endocrine control, in response to osmotic stimulii, earlier studies of Schmidt-Neilsen and his co-workers indicated that the gland was under the control of the parasympathetic nervous system. In fact, by electrically stimulating a branch of the trigeminal nerve, the ramus opthalmicus, which innervates the paired supra-orbital glands of the herring gull (Larus argentatus) an extra-renal excretory response was elicited (12). Such a response, however, may have been associated with vasomotor changes in the gland and may not have been involved in the active transport of electrolytes.

This investigation then, was concerned with one of these extra-renal excretory systems; namely, the supra-orbital glands of the Domestic duck. The similarity in structure and function of these glands to the vertebrate kidney and the fact that the vertebrate kidney is to large extent under endocrine control, lead us to consider the possibility that these glands are under the influence of adrenocortical steroids (12, 13, 14, 15, 16).

Materials and Methods

I. All birds used throughout these experiments were nine to twelve week old male domestic ducks of the Peking white variety. They were fed daily wih commercial "duck grower food" and given an ad libidum supply of fresh water. The ten control birds and each experimental group of five animals were starved for twenty-four hours before use. A wing vein was cannulated and the bird was heparinized one and one half hours prior to the experiment. A sheet of polythene was then placed under the cloacal region and shaped in the form of a funnel. The bird was fastened in a prone position to a standard "dog board" and a hole was cut in the "funnel" for urine collection. The animal was in such a position that blood, urine, and nasal samples could be easily taken.

Saline loading was by means of a flexible plastic tube connected to a syringe. Twenty ccs. of 20% NaCl was introduced directly into the proventriculus. The time of saline loading was taken to be zero time for all experiments.

A control blood sample was taken from the cannulated wing vein prior to saline loading and subsequent to loading samples were taken at $\frac{1}{2}$ hour intervals for the first three hours and at hourly intervals for the remaining experimental period. The blood samples were cooled to 5 degrees centigrade and immediately centrifuged at 5000 rpm. for five minutes. Urine samples were collected at twenty minute intervals after zero time and the volume of urine was measured. Those samples that were contaminated with feces were discarded as it was found that they contained disproportionate amounts of potassium. The time of onset of nasal secretion was recorded, and collections were made over 45 minute intervals thereafter. The samples were collected in silica coated 250 ml beakers.

The cortisol and cortexone treated birds received 5 mg intramuscular doses 12 hours and $1\frac{1}{2}$ hours prior to saline loading. Both these steroids were free alcohol preparations in stabilized suspension. ACTH (Acton X, Nordic Biochemicals

Ltd.) was administered daily for five days prior to the experiment, each bird receiving 6 doses of 20 I.U. in isotonic saline. The dl aldosterone (Ciba) was in oil suspension and 250 ug. doses were given 1½ and zero hours before loading.

Adrenalectomy was performed via a dorso-lateral incision between the second and third ribs. Bilateral adrenalectomy was done in two successive stages on successive days. The birds were used experimentally 6 to 12 hours after removal of the second gland. Unilateral adrenalectomy was performed approximately 12 hours before saline loading. After this experimental period the unilaterally adrenalectomized animals were intra-muscularly injected with 10 mg of cortisol in stabilized suspension and the second adrenal gland was removed. These totally adrenalectomized ducks were then maintained for 4 days with daily intra-muscular injections of 5 mg. of cortisol. The saline loading was then repeated with these birds but serial blood samples were not taken.

II. Flame Photometric Techniques:

Blood:

The blood plasma was pipetted with 0.5 cc. T.C. pipettes directly into 50 ml volumetric flasks whereupon 0.25 cc of HCl and 1 drop of caprylic alcohol were added to bring the volume to 50 ccs. The solution then was poured into Petrie dishes and read against a standard solution of known concentration on a Zeiss PF5 flame photometer. Sodium was read against a 10 mg% standard solution with proportionate amounts of potassium and calcium added to correct for interference. Conversely the 0.5 mg% potassium standard contained sodium and calcium. The caprylic alcohol was added to prevent foaming.

Urine:

The urine samples were collected in 500 cc. beakers after which the volume of the sample was measured and recorded. Aliquots of 0.5 cc. were pipetted into 100 cc. volumetric flasks then 1 drop of caprylic alcohol was added. The urine

solution was then read against the same standard used for the plasma samples. Urine sodium and potassium concentrations were then calculated.

Nasal Samples:

As nasal samples were collected in beakers for periods of 45 minutes, an evaporation error was incurred when the volume of small samples was measured. To correct for this error the sample was pipetted into a 100 cc. volumetric flask then the crystalline salt was washed with distilled water into the volumetric flask with the initial sample. In this way we obtained an approximate value for volume as well as an accurate measure of the total output of salt during a 45 minute time interval. From the 100 cc. sample an aliquot of from 1 to 10 cc. was transferred to another 100 cc. volumetric flask and diluted to volume. This sample was read against the serum sodium and potassium standards. All electrolyte values were expressed as milliequivalents per litre.

Results

The dominant characteristic of these experiments was the diphasic response to a saline load. All birds showed an initial diuresis during the first hour after loading and at varying times during this phase the onset of nasal secretion occurred.

I. Renal phase Table I Figures 1 and 2

a) Intact Controls

The control birds showed a mean total diuresis of 51.9 ± 3.2 cc. during the three 20 minute collection periods immediately after salt loading. The volume of urine excreted during these periods progressively declined from 23.3 ± 4.8 cc. to 11.5 ± 4.2 cc. The disproportionate decline in the Na^+ content of these samples was reflected in the significant fall in concentration from 633.0 ± 70.4 to 248.0 ± 115.6 m.eq./litre ($p < .01$). Conversely the potassium concentration rose from 13.6 ± 2.8 to 24.7 ± 12.2 m.eq./litre in the same samples, but this change was not statistically significant.

b) Intact and Cortisol

Cortisol treatment did not reduce the initial diuresis, indeed the mean total volume was slightly greater. The total sodium output during this diuresis was, however, less than the control value due to the fact that the first sample contained 7.9 ± 1.5 m.eq. in 24.3 ± 2.6 cc. whereas the first control sample contained 14.8 ± 6.2 m.eq. in 23.3 ± 4.8 cc. A trend toward a diminishing Na^+ concentration of the individual samples was also apparent after cortisol treatment. As in the case of the control birds the pattern of potassium excretion was not as clear-cut. The total output of 0.99 ± 0.18 m.eq. was not significantly greater than the control value of 0.71 ± 0.23 m.eq. Examination of the Na/K ratios for both excretion and concentration showed a significant decline compared to the corresponding control values ($p < .01$).

Legend for Table I. The analysis of successive 20 minute urine samples from adrenocortical steroid treated, totally and subtotally adrenalectomized ducks subsequent to hypertonic saline loading.

	Urine Sample	Volume in cc.	Total Renal Output in m.eq. Na^+	K^+	$Na^+ : K^+$	Urine Concentration in m.eq./litre Na^+	K^+
ONTROL	1	23.3 ±4.8	14.8 ±6.2	0.27 ±0.02	49.2 ±6.3	633.0 ±70.4	13.6 ±2.8
	2	17.1 ±4.3	7.7 ±1.8	0.17 ±0.06	52.1 ±9.2	455.8 ±34.8	11.0 ±7.7
	3	11.5 ±4.2	2.4 ±1.6	0.27 ±0.23	46.3 ±16.5	248.0 ±115.6	24.7 ±12.2
	Total	51.9 ±3.2	24.9 ±2.9	0.71 ±0.23	47.4 ±8.8	482.2 ±24.1	12.7 ±3.5
ORTISOL	1	24.3 ±2.6	7.9 ±1.5	0.48 ±0.09	18.9 ±2.8	327.0 ±31.9	16.1 ±2.9
	2	23.3 ±0.9	6.5 ±0.7	0.26 ±0.002	24.8 ±2.7	265.6 ±17.3	11.5 ±1.1
	3	12.2 ±6.4	2.2 ±1.8	0.25 ±0.10	7.3 ±3.8	179.8 ±67.4	36.7 ±14.9
	Total	59.8 ±7.9	16.6 ±2.4	0.99 ±0.18	17.1 ±2.1	278.1 ±23.2	17.8 ±2.9
ORTEXONE	1	30.3 ±5.3	20.6 ±3.4	0.33 ±0.05	62.1 ±1.3	682.2 ±83.4	10.9 ±0.9
	2	8.5 ±3.2	3.0 ±1.1	0.06 ±0.002	52.4 ±4.2	358.3 ±3.3	7.1 ±0.4
	3	9.9 ±3.7	1.5 ±1.2	0.66 ±0.24	2.1 ±1.4	158.0 ±109.6	61.5 ±12.6
	Total	48.7 ±5.8	25.1 ±3.1	1.05 ±0.26	34.2 ±8.4	520.3 ±15.2	11.8 ±5.3
LDOSTERONE	1	11.6 ±3.0	4.6 ±1.6	0.19 ±0.04	25.9 ±6.8	389.1 ±67.6	19.3 ±5.9
	2*	2.5	1.2	0.01	23.3	467.8	5.8
	3	-	-	-	-	-	-
	Total	14.1 ±4.9	5.8 ±2.4	0.20 ±0.05	28.8 ±9.7	361.1 ±68.4	18.3 ±6.2

	Urine Sample	Volume in cc.	Total Renal Output in m.eq. Na^+	K^+	$Na^+ : K^+$	Urine Concentration in m.eq./litre Na^+	K^+
ACTH	1	35.6 ±2.9	20.3 ±2.5	0.43 ±0.004	47.0 ±5.5	576.0 ±49.2	12.7 ±1.4
	2	18.6 ±4.3	7.3 ±2.1	0.15 ±0.01	51.5 ±9.9	394.8 ±60.6	7.8 ±0.3
	3	5.9 ±3.2	2.0 ±1.1	0.04 ±0.02	71.2 ±21.3	380.0 ±18.9	5.9 ±1.6
	Total	60.1 ±6.8	29.6 ±2.7	0.62 ±0.02	49.1 ±3.3	503.9 ±18.9	10.3 ±0.5
UNILATERAL ADRENALECTOMY	1	19.5 ±11.1	11.8 ±3.9	0.84 ±0.53	16.4 ±5.6	683.3 ±67.4	38.9 ±5.4
	2	23.0 ±5.8	8.3 ±2.8	0.28 ±0.07	36.4 ±9.2	339.6 ±48.1	11.76 ±2.5
	3	1.2*	0.1	0.20 ±0.11	1.5	91.7	59.5
	Total	42.7 ±12.1	20.3 ±5.9	1.32 ±0.52	20.2 ±5.3	398.5 ±69.2	29.8 ±7.8
TOTAL ADRENALECTOMY	1	22.9 ±8.5	14.7 ±6.5	0.44 ±0.19	32.3 ±8.2	595.9 ±79.5	32.3 ±14.4
	2	25.8 ±6.8	13.6 ±4.7	0.33 ±0.12	50.9 ±9.8	505.6 ±52.1	12.3 ±3.5
	3	10.5 ±4.5	3.6 ±1.5	0.13 ±0.06	37.6 ±16.4	398.3 ±84.4	8.7 ±1.2
	4+	23.1 ±9.6	2.5 ±2.0	0.18 ±0.08	13.3 ±2.3	65.7 ±15.8	7.5 ±0.5
	Total	82.3 ±4.8	34.4 ±3.5	1.08 ±0.07	32.8 ±4.5	478.3 ±85.7	16.7 ±4.2
TOTAL ADRENALECTOMY + CORTISOL	1	37.1 ±12.9	16.9 ±5.5	0.43 ±0.15	45.7 ±11.6	564.0 ±184.7	12.4 ±2.0
	2	8.1 ±4.4	1.6 ±0.5	0.15 ±0.10	27.6 ±15.2	221.4 ±83.1	14.7 ±3.8
	3	-	-	-	-	-	-
	4*	1.8*	0.2	0.04	4.3	95.6	21.0
	Total	46.0 ±9.4	18.7 ±5.1	0.62 ±0.02	34.7 ±12.2	437.8 ±153.0	14.8 ±2.6

c) Intact and Cortexone

Treatment with cortexone did not significantly alter the total diuresis although there was a 70% reduction in volume between the first and third samples compared with a 50% decline in the controls. Similarly, there was no significant change in the total sodium excreted when compared to the controls but there was a significant fall from 20.6 ± 3.4 m.eq. in the first sample to 1.5 ± 1.2 m.eq. in the third sample ($p < .01$). This represented a 92% reduction compared to 83% in the controls. Therefore, although there was an increased reabsorption of water under the influence of cortexone there did not appear to be a commensurate increase in the tubular reabsorption of sodium. Comparison of the potassium content of the first and third urine samples showed an apparent rise from 0.33 ± 0.05 m.eq. to 0.66 ± 0.24 m.eq. but this increase was not significant. This trend was reflected in the urine concentrations of sodium and potassium and in the Na/K ratios.

d) Intact and Aldosterone

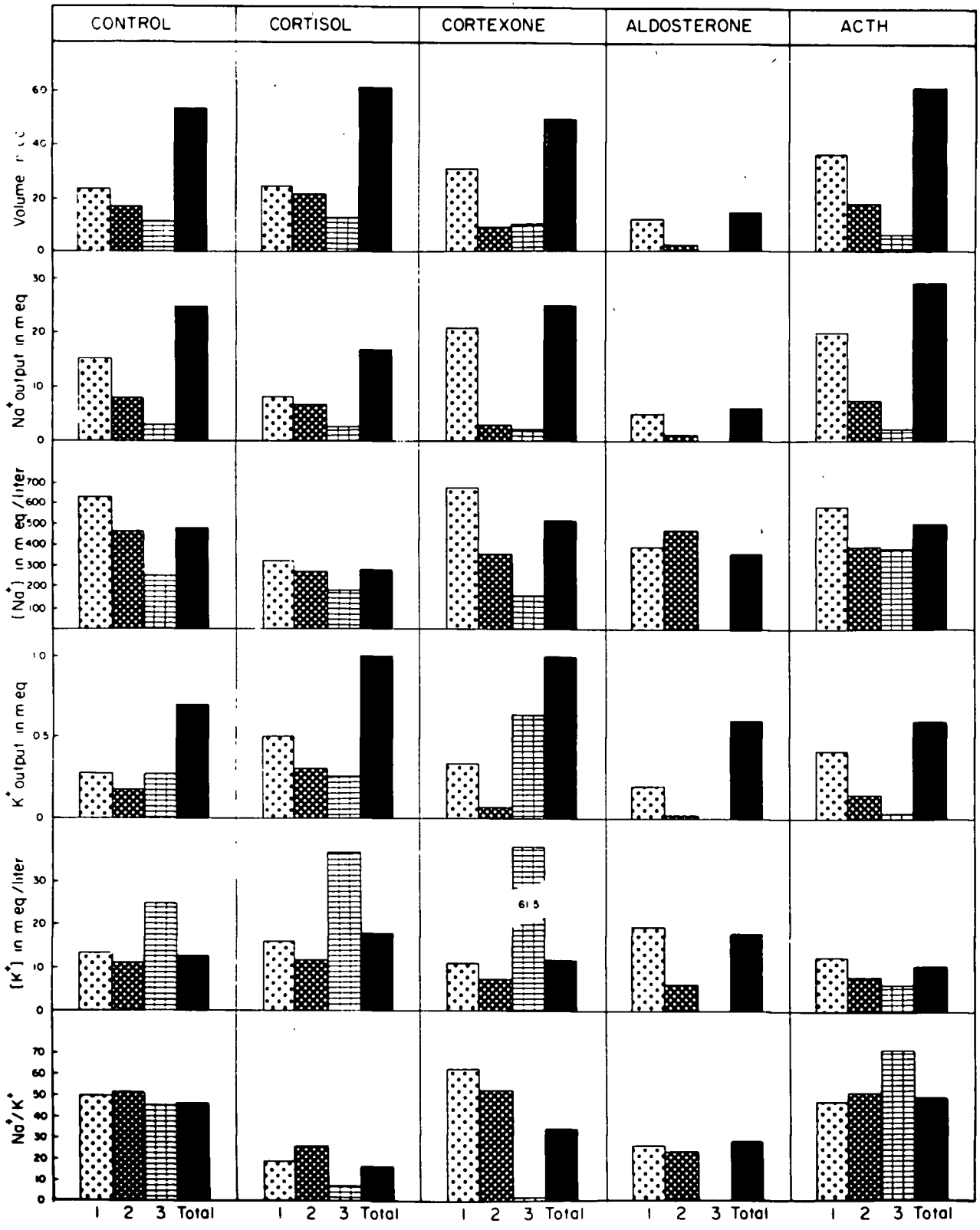
There was a marked reduction in the diuretic phase of the aldosterone treated birds. Only one bird urinated during the second sample period and none during the third. Consequently the total volume excreted (14.1 ± 4.9 cc.) was significantly lower than any other group ($p < .001$).

The total amount of sodium excreted was 5.8 ± 2.4 m.eq. in a mean volume of 14.1 ± 4.9 cc. This concentration of 361.1 ± 68.4 represented a considerable decline in renal sodium excretion when compared to the control birds. Although the total amount of potassium excreted was significantly less than the control value (0.71 ± 0.23 V 0.20 ± 0.05 m.eq.) there was, however, no trend towards an increase in the potassium concentration of these urines (19.3 ± 5.9 V 18.3 ± 6.2 m.eq./litre).

e) Intact and ACTH

As in the case of cortisol treatment, ACTH slightly increased the total volume of urine excreted during the first hour, but although these volumes were

Legend for Figure 1. A histogram showing the comparative analysis of the volume and electrolyte composition of 20 minute urine samples from adrenocortical steroid treated ducks subsequent to hypertonic saline loading.


CONTROL
CORTISOL
CORTEXONE
ALDOSTERONE
ACTH
Volume
Na+ output in m eq
[Na+] in m eq/liter
K+ output in m eq
[K+] in m eq/liter
Na+/K+
61.5
1 2 3 Total

virtually identical (59.9 V 60.7 cc.) the ACTH group excreted approximately 80% more sodium than the cortisol group. This was reflected in the sodium concentrations of the successive urine samples. Indeed, the total sodium excreted after treatment with ACTH (29.6 ± 2.7 m.eq.) appeared higher than both the cortisol (16.7 ± 2.4 m.eq.) and the control (24.9 ± 2.9 m.eq.) values, but the difference was only significant when compared to cortisol ($p < .01$). The urine sodium concentrations were in no case higher than the corresponding control values. The potassium output in the three urine samples declined as in the case of the control and cortisol groups.

f) Sub-total adrenalectomy

Unilateral adrenalectomy 12 hours prior to saline loading did not have any significant effect on either the total volume of urine or the total sodium and potassium excreted. The phase of diuresis, however, was shorter in 4 of the birds; there being a third urine sample in one duck.

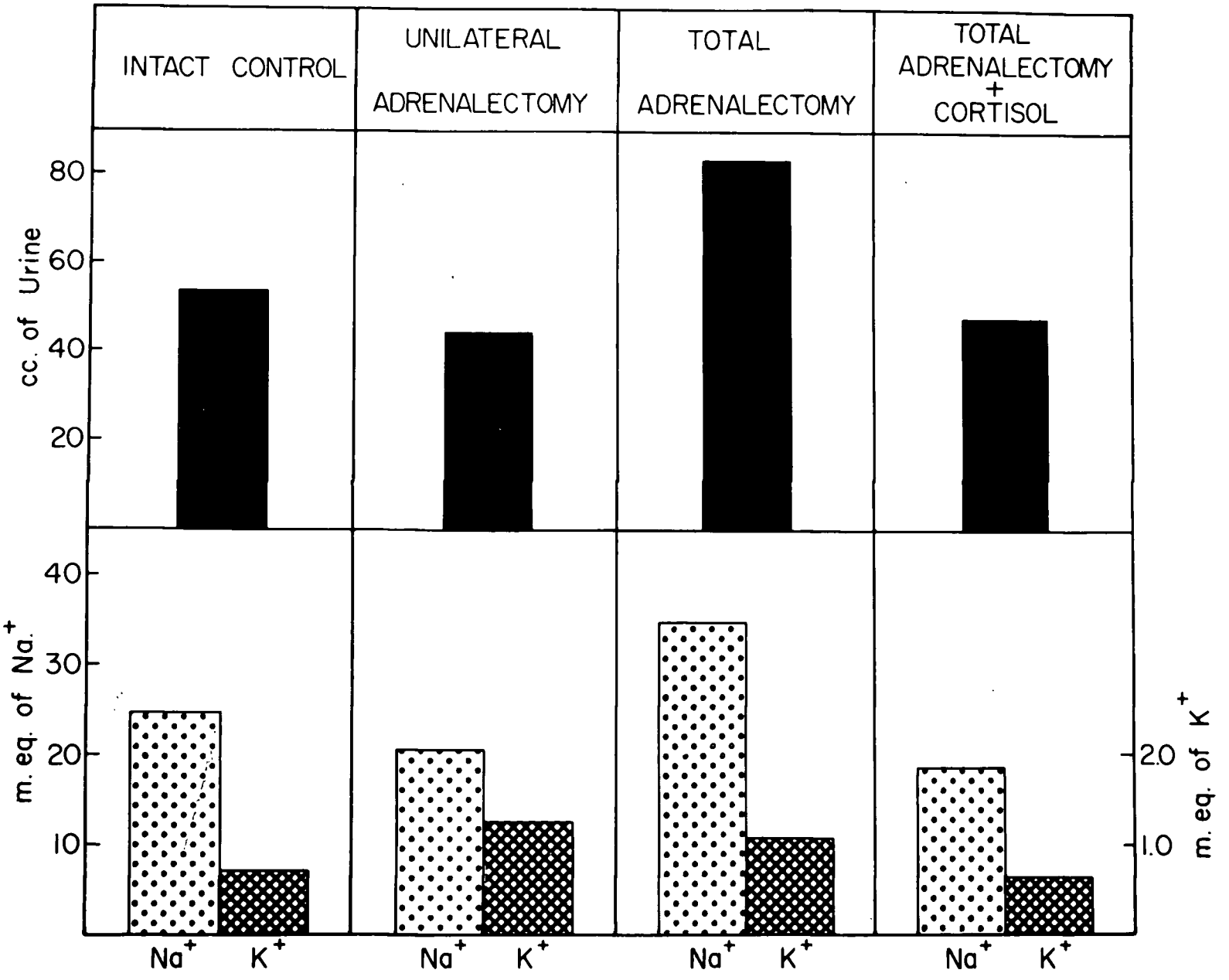
g) Total adrenalectomy

Total adrenalectomy considerably extended the period of diuresis subsequent to saline loading. The degree of extension varied from 1 to 15 twenty minute urine samples with a mean number of 6.6 samples for the five birds in the group. The results of the analysis of urine samples from the fourth collection period and later have been pooled for each bird and are indicated as mean values of sample "4+" in Table I. The total volume of urine excreted by the adrenalectomized ducks was 82.3 ± 4.8 cc. which was significantly higher than the control value of 51.9 ± 3.2 cc. ($p < .001$). The total amount of sodium excreted was 34.4 ± 3.5 m.eq. which was higher than the control output at somewhat less than the 10% level of significance ($p < .10 > .05$). Potassium excretion appeared to be unchanged when compared to the controls.

h) Total Adrenalectomy and Cortisol

Maintenance of the adrenalectomized birds with exogenous cortisol significantly

Legend for Figure 2. The renal response of the Domestic duck (*Anus platyrhynchus*) to a hypertonic saline load after total and subtotal adrenalectomy - a comparison of the total volume, sodium and potassium excreted.


INTACT CONTROL
UNILATERAL ADRENALECTOMY
TOTAL ADRENALECTOMY
TOTAL ADRENALECTOMY + CORTISOL
cc. of Urine
80
60
40
20
m. eq. of Na+
40
30
20
10
m. eq. of K+
2.0
1.0
Na+
K+
Na+
K+
Na+
K+
Na+
K+

reduced the volume of urine excreted to 46.0±9.4 cc. This value was significantly lower than the high rate of diuresis recorded for the adrenalectomized birds ($p < .02 > .01$) but did not differ significantly from the controls ($p < .5$). Similarly there was a significant renal retention of sodium since the total output was 18.7±5.1 m.eq. compared to 34.4±3.5 m.eq. after total adrenalectomy ($p < .05, > .025$). This total sodium output, however, did not vary significantly from the control output ($p < 0.2$). No significant effect of cortisol could be demonstrated with respect to potassium excretion.

II. Extra-renal phase Tables II, III and IV, Figures 3, 4, 5 and 6

a) Intact Controls

The initial rate of nasal secretion for the control birds during the first collection period was 1.58±0.42 cc./45 min. and this rate of output increased to a maximum of 4.95±0.56 cc. during the third period. The subsequent decline in output resulted in complete cessation during the final period i.e., 315 to 360 minutes after loading. The concentrations of sodium and potassium in the nasal fluid did not differ between successive samples and the total outputs in the 28.9±3.5 cc. discharged were 17.1±1.6 and 0.44±0.06 m.eq. respectively indicating mean concentrations of 602.6±13.9 and 17.3±0.49 m.eq./litre. The ratios of extra-renal to renal excretion of sodium and potassium were 0.85±0.22 and 0.94±0.30 respectively.

b) Intact and Cortisol

After cortisol treatment there was a significant increase in the initial rate of nasal discharge 1.58±0.42 V 5.34±0.84 cc./45 min. This increase continued up to a maximum between 180 and 225 minutes after loading and subsequently a decline occurred which continued throughout the remainder of the experiment. None of the cortisol treated birds ceased to drip during the experimental period and the mean total output of 59.0±5.1 cc. was significantly greater than the corresponding

Legend for Table II. A comparison of the times of onset of nasal secretion and the concurrent plasma levels of sodium in adrenocortical steroid treated, total and subtotally adrenalectomized ducks, after hypertonic saline loading.

GROUP	Time of onset of nasal secretion mins.	"p" Value	Initial rise in serum Na^+ m.eq./litre/hr.	"p" Value	Serum Na^+ at onset of nasal secretion m.eq./litre	"p" Value
CONTROLS	52.8 ±11.3	-	28.7 ±2.3	-	160.6 ±2.5	-
CORTISOL	16.0 ±6.5	<.02 >.01	31.8 ±2.9	<.4	153.9 ±2.4	<.1 >.2
CORTEXONE	24.0 ±6.5	<.05 >.10	35.6 ±3.3	<.2 >.1	159.2 ±2.6	<.8
ALDOSTERONE	14.0 ±3.3	<.01 >.001	30.0 ±5.3	<.8	152.0 ±1.2	<.01 >.001
ACTH	11.4 ±1.9	<.01 >.001	34.6 ±2.6	<.2 >.1	151.8 ±1.6	<.01 >.001
UNILATERAL ADX	116.6 ±9.4	<.001	28.4 ±4.8	<.9	170.2 ±3.4	<.05 >.02
TOTAL ADX	270*	-	32.8 ±3.3	<.3 >.2	176.0	-
TOTAL ADX + CORTISOL	30.2 ±14.8	<.3 >.2	28.7** ±2.3	-	155.7** ±3.4	<.3 >.2

* Only one bird of the group showed any nasal secretion.

** Intact control serum values used for these calculations.

Legend for Table III. The volume and electrolyte composition of the nasal fluid after the hypertonic saline loading of intact, adrenocortical steroid treated, subtotally and totally adrenalectomized ducks maintained on cortisol.

GROUP	TOTAL OUTPUT				CONCENTRATIONS	
	Volume ccs.	Na^+ m.eq.	K^+ m.eq.	$Na^+ : K^+$ ratio	Na^+ m.eq./liter	K^+ m.eq./liter
CONTROL	28.9 ±3.5	17.1 ±1.6	0.44 ±0.06	36.8 ±2.8	602.6 ±13.9	17.3 ±0.49
CORTISOL	59.0** ±5.1	31.3** ±2.4	0.89** ±0.06	35.6 ±0.8	534.0* ±14.8	15.2** ±0.40
CORTEXONE	54.6** ±4.6	27.9** ±2.8	0.92** ±0.09	31.2 ±2.8	511.2** ±11.9	16.8 ±1.10
ALDOSTERONE	82.1** ±3.8	45.9** ±2.2	1.08** ±0.04	42.8 ±0.8	560.4* ±13.8	13.2** ±0.56
ACTH	40.3* ±4.2	21.5 ±1.7	0.66 ±0.10	35.7 ±1.6	541.9* ±19.8	15.3* ±0.81
UNILATERAL ADX	15.5* ±3.9	8.6** ±2.2	0.21* ±0.05	32.3 ±7.4	571.3 ±25.0	14.4* ±1.2
TOTAL ADX	-	-	Trace	-	-	-
TOTAL ADX + CORTISOL	48.1 ±11.4	19.5 ±7.1	0.63 ±0.17	38.5 ±3.9	486.9** ±24.1	13.09** ±1.0

* $p < .05 > .01$ with respect to corresponding control value.

** $p < .01$ with respect to corresponding control value.

Legend for Table IV. The volumes of nasal fluid collected from saline loaded, adrenocortical treated, totally and subtotally adrenalectomized ducks during 45 minute intervals after the onset of nasal activity.

GROUP	Body Wt. Kg.	(0–45) 1	(45–90) 2	(90–135) 3	(135–180) 4	(180–225) 5	(225–270) 6	(270–315) 7	(315–360) 8	(360–405) 9	(405–450) 10	(450–495) 11	Vol. cc.	"p" value*
CONTROL	2.54 ±0.04	1.58 ±0.42	4.75 ±0.61	4.95 ±0.56	4.79 ±0.70	3.77 ±0.61	2.89 ±0.67	2.45 ±0.56	1.42 ±0.48	1.08 ±0.44	0.97 ±0.34	0.28 ±0.15	28.9 ±3.6	
CORTISOL	2.66 ±0.09	5.34 ±0.84	6.09 ±1.04	6.41 ±0.93	7.27 ±0.90	7.26 ±1.09	7.40 ±0.99	5.75 ±0.87	4.44 ±0.52	4.16 ±0.52	3.02 ±0.49	1.84 ±0.32	59.0 ±4.9	<.005 >.001
CORTEXONE	2.74 ±0.11	4.92 ±0.62	7.11 ±1.27	8.08 ±0.98	7.38 ±1.25	6.33 ±0.68	5.98 ±1.00	5.05 ±1.35	3.49 ±0.88	2.95 ±0.88	2.42 ±1.04	0.92 ±0.35	54.6 ±4.2	<.01 >.005
ALDOSTERONE	2.46 ±0.05	5.04 ±2.05	7.09 ±1.29	9.57 ±1.55	9.94 ±1.67	9.87 ±1.41	9.62 ±1.11	9.02 ±1.39	8.81 ±2.00	6.29 ±1.41	4.37 ±1.41	2.54 ±0.72	82.1 ±3.8	<.001
ACTH	2.42 ±0.06	6.18 ±1.41	7.14 ±0.85	7.80 ±0.88	7.60 ±0.90	5.52 ±0.45	3.14 ±0.75	1.44 ±0.45	0.79 ±0.31	0.58 ±0.33	0.11 ±0.10	-	40.3 ±4.2	<.025 >.01
UNILATERAL ADX	2.44 ±0.05	4.35 ±1.01	3.85 ±1.02	3.12 ±1.69	1.86 ±0.85	0.93 ±0.83	0.89 ±0.79	0.45 ±0.39	-	-	-	-	15.5 ±3.9	<.02 >.01
TOTAL ADX	2.38 ±.04	-	-	-	-	-	-	Transient secretion by one bird						
TOTAL ADX + CORTISOL	2.44 ±0.05	3.81 ±0.5	8.06 ±1.11	8.17 ±1.08	8.86 ±1.43	6.26 ±1.50	4.52 ±2.00	3.71 ±2.29	2.98 ±2.58	1.76 ±1.50	-	-	48.1 ±11.4	<.2 >.1

* "p" value with respect to control total volume.

** only one bird of the group showed any nasal secretion.

control volume ($p < .01$). The sodium concentration of the nasal fluid, however, was lower than the control and this decrease was significant. On the other hand, due to the enhanced nasal volume, the total sodium output was higher in the cortisol treated ducks ($p < .01$). The potassium concentration was similar to the control value and represented approximately 3% of the sodium concentration. The extra-renal:renal ratio for sodium was 2.08 ± 0.29, which reflected the simultaneous extra-renal enhancement and renal depression of sodium output. The value for potassium did not differ significantly from the controls (0.97 ± 0.17 V 0.94 ± 0.30).

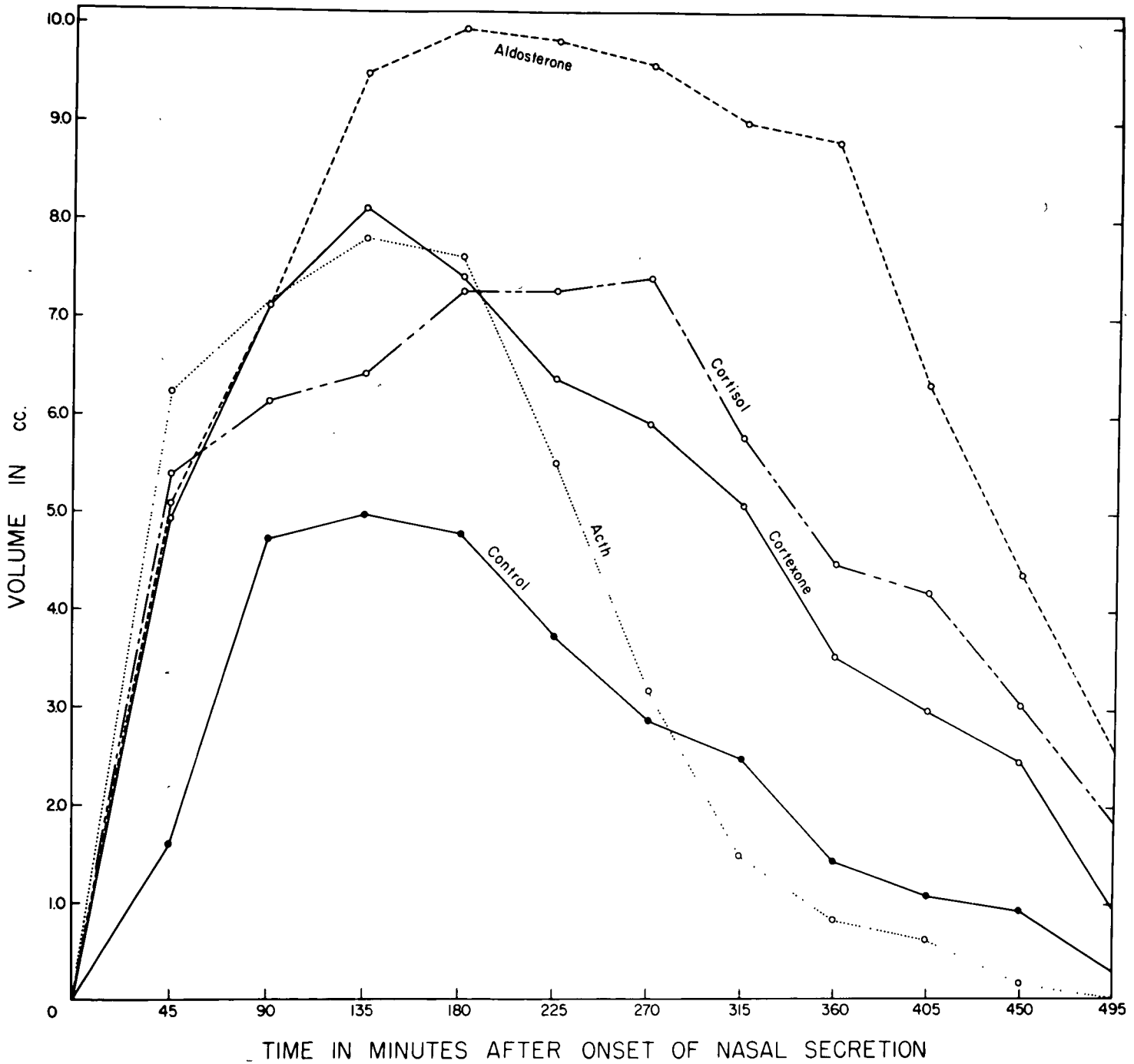
c) Intact and Cortexone

The effect of cortexone on the nasal secretion after salt loading appeared to be less than that observed for cortisol. Nevertheless, the mean initial rate of output (4.92 ± 0.62 cc./45 min.) and the mean total volume secreted (54.6 ± 4.6 cc.) were significantly higher than the control values ($p < .01$). The maximum rate of nasal secretion was between 135 and 150 minutes after loading which coincided with the control birds but preceeded the cortisol treated animals. As in the case of cortisol the sodium concentration of the nasal discharge was significantly lower than the controls ($p < .01$) although the potassium concentration was the same, but due to the enhanced volume secreted the total output of sodium and potassium were significantly greater than the controls ($p < .01$).

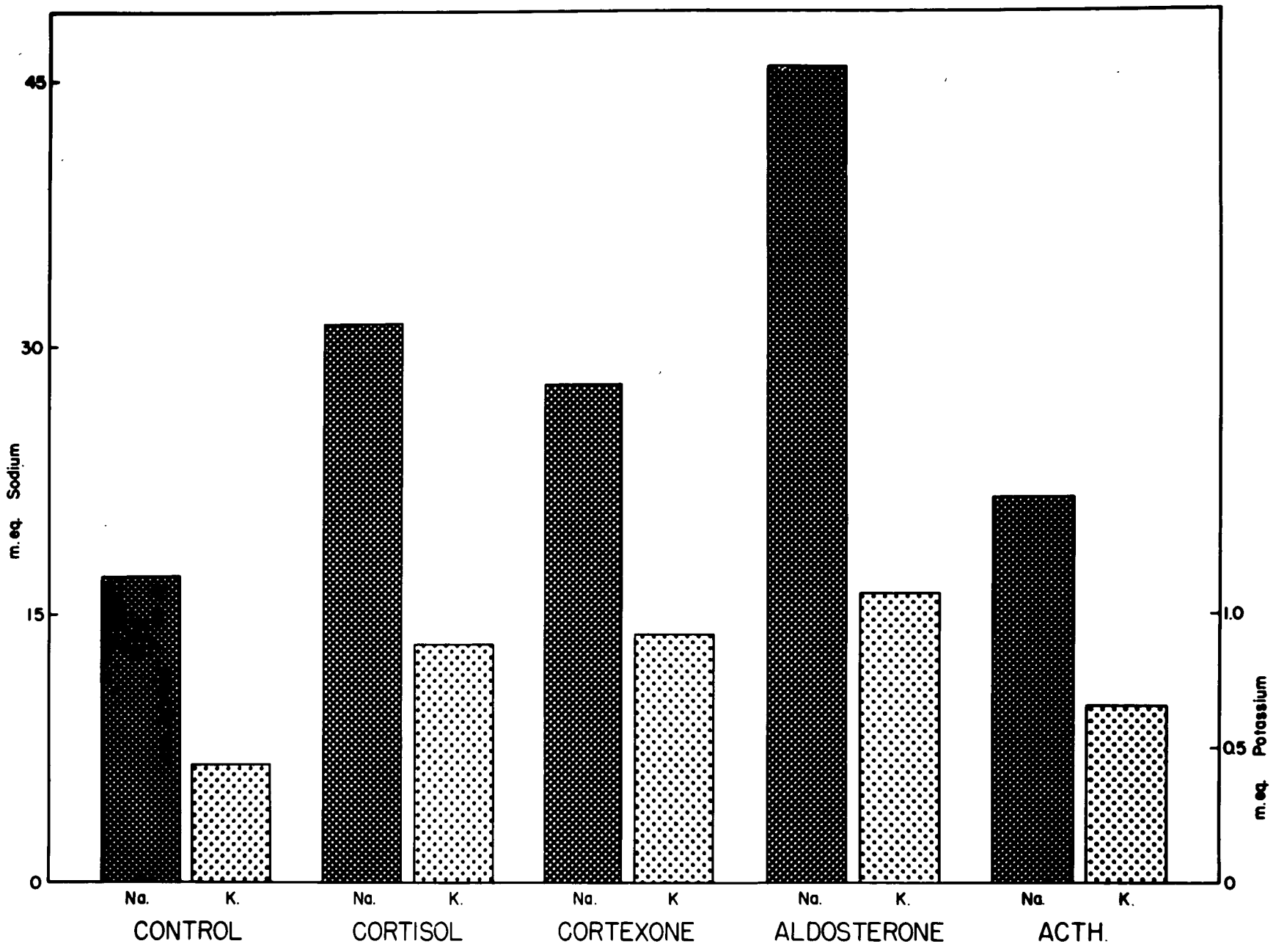
d) Intact and Aldosterone

Treatment with aldosterone caused an initial nasal secretion of 50.4 ± 2.05 cc./45 min. which, during the period from 135 to 180 minutes after loading, increased to 9.94 ± 1.67 cc. This volume and the total nasal output of 82.1 ± 3.8 cc. were the highest volumes recorded in any of these experiments. Again the mean sodium concentration of the nasal secretion was significantly lower than the control birds ($p < .05$) but the total extra-renal sodium output (45.9 ± 2.2 m.eq.) was higher due to the increased volume ($p < .01$). Similarly the total potassium output was

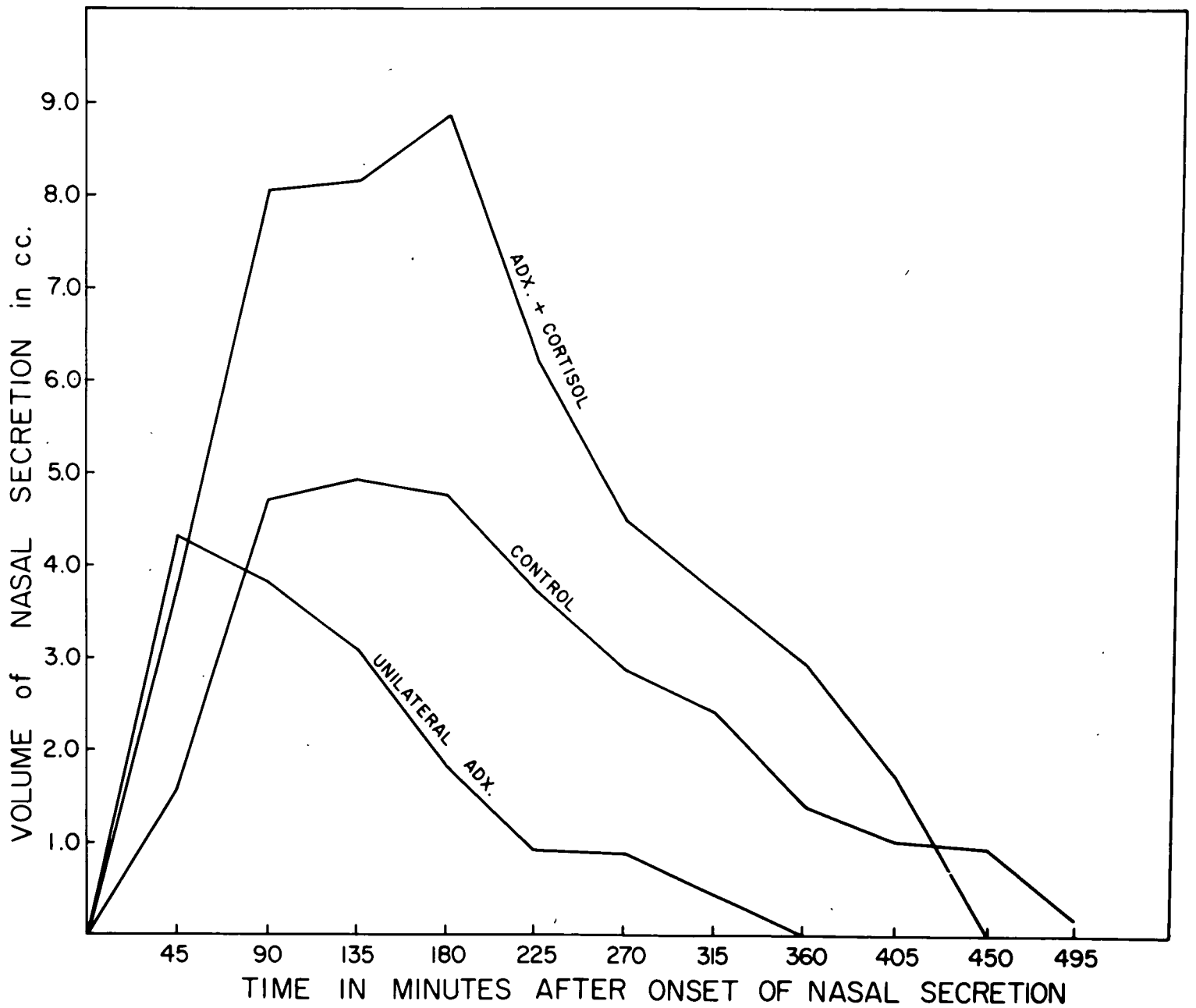
Legend for Figure 3. The accumulative output of nasal fluid after hypertonic saline loading in Domestic ducks following treatment with adrenocortical steroids.


10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
VOLUME IN cc.
Aldosterone
Corlisol
Cortexone
Acth
Control
45
90
135
180
225
270
315
360
405
450
495
TIME IN MINUTES AFTER ONSET OF NASAL SECRETION

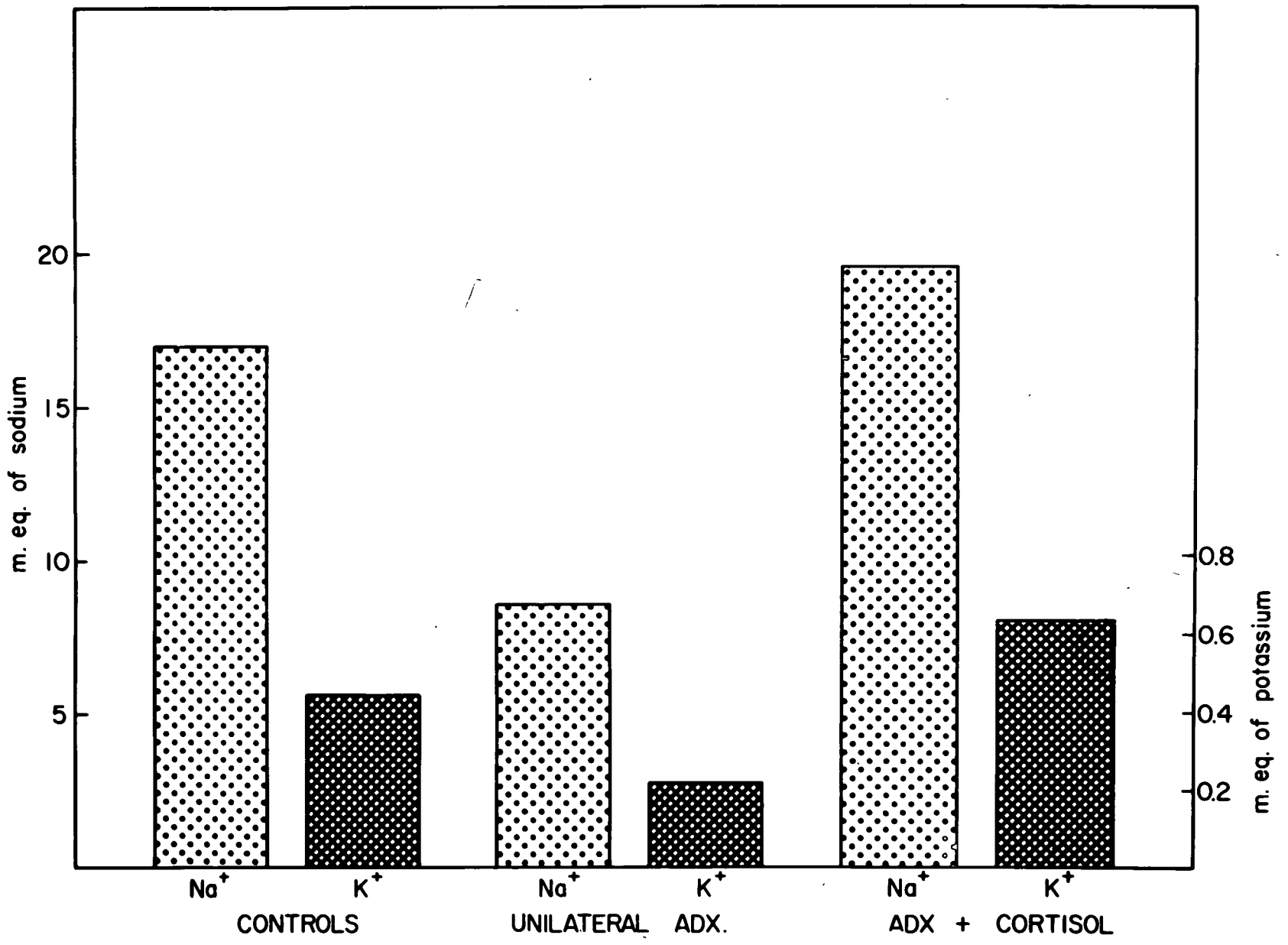
Legend for Figure 4. A comparison of the extra-renal electrolyte output of salt loaded ducks after treatment with adrenocorticoid steroids.


45
30
15
0
m.eq. Sodium
1.0
0.5
0
m.eq. Potassium
Na.
K.
CONTROL
CORTISOL
CORTEXONE
ALDOSTERONE
ACTH.

Legend for Figure 5. The accumulative output of nasal fluid after the hypertonic saline loading of intact, subtotally adrenalectomized ducks and totally adrenalectomized ducks maintained on cortisol.


VOLUME of NASAL SECRETION in cc.
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ADX. + CORTISOL
CONTROL
UNILATERAL ADX.
45
90
135
180
225
270
315
360
405
450
495
TIME IN MINUTES AFTER ONSET OF NASAL SECRETION

Legend for Figure 6. A comparison of the extra-renal electrolyte outputs, in response to saline loading, from control, unilaterally adrenalectomized and totally adrenalectomized ducks maintained on cortisol.


m. eq. of sodium
20
15
10
5
m. eq. of potassium
0.8
0.6
0.4
0.2
Na+
K+
Na+
K+
Na+
K+
CONTROLS
UNILATERAL ADX.
ADX + CORTISOL

significantly higher in spite of a decline in the concentration from 17.3 ± 0.49 to 13.2 ± 0.56 m.eq./liter compared to the controls ($p < .01$). Examination of the nasal:urine ratios showed a value for sodium which was twice that observed with respect to volume and potassium. This was least in part due to the high renal tubular reabsorption of sodium and the simultaneous enhancement of nasal secretion. Cortisol was the only other hormone which brought about this disproportionate ratio for sodium.

e) Intact and ACTH

ACTH induced a significant increase in the total nasal volume secreted (40.3 ± 4.2 cc.) and the initial rate of output was the highest recorded (61.8 ± 1.41 cc./45 min.). The period of response, however, was somewhat shorter than the control birds since the decline in secretion was more rapid. The nasal sodium concentration of 541.9 ± 19.8 m.eq./litre was significantly lower than the controls ($p < .05$) and there was consequently a disproportionately lower increase in the total extra renal sodium secreted compared to the controls (21.5 ± 1.7 V 17.1 ± 1.6 m.eq.).

f) Sub-total Adrenalectomy

During the first 45 minute collection period the unilaterally adrenalectomized ducks showed a nasal output of 4.35 ± 1.01 cc. This initial output was the highest for any collection period and was significantly higher than the corresponding control value ($p < .05 > .02$) however there was a decline in output after the first 45 minutes resulting in complete cessation in all birds 225 mins. after loading. The mean total output of 15.5 ± 3.9 cc. contained 8.6 ± 2.2 m.eq. of sodium and 0.21 ± 0.05 m.eq. of potassium. All these values were significantly lower than the controls ($p < .05, > .01$ and $< .02$ respectively). The concentrations of sodium and potassium were not statistically significantly different from the controls.

g) Total adrenalectomy

The totally adrenalectomized animals showed a complete cessation of

Legend for Table V. The changes in serum sodium and potassium concentrations in adrenocortical steroid treated, totally and subtotally adrenalectomized ducks after hypertonic saline loading.

Serum electrolyte concentration in m.eq./litre at time in minutes after saline load.

		0	30	60	90	120	150	210	270	330	390	450	510
CONTROL	Na^+	144.1 ±0.9	158.4 ±0.7	163.5 ±2.1	166.5 ±2.5	167.5 ±2.7	168.9 ±2.7	169.3 ±2.9	167.5 ±3.0	167.1 ±2.4	168.1 ±2.1	166.4 ±2.3	165.6 ±2.1
	K^+	3.43 ±0.07	3.42 ±0.08	3.71 ±0.06	3.92 ±0.07	4.02 ±0.06	4.18 ±0.06	4.17 ±0.06	4.07 ±0.08	3.93 ±0.07	3.86 ±0.07	3.72 ±0.06	3.79 ±0.08
CORTISOL	Na^+	145.3 ±1.3	163.2 ±1.9	165.4 ±2.1	169.2 ±1.4	172.6 ±1.3	173.0 ±1.5	172.8 ±1.7	170.0 ±1.7	168.8 ±2.1	166.8 ±2.4	166.0 ±2.6	164.6 ±1.9
	K^+	3.89 ±0.10	3.97 ±0.08	4.14 ±0.04	4.22 ±0.11	4.30 ±0.06	4.46 ±0.04	4.54 ±0.04	4.24 ±0.18	4.48 ±0.12	4.22 ±0.08	4.06 ±0.06	3.92 ±0.10
CORTEXONE	Na^+	146.6 ±1.5	164.4 ±2.0	167.0 ±1.5	171.2 ±1.8	172.0 ±1.0	172.0 ±1.0	170.8 ±0.7	170.0 ±0.6	168.8 ±0.4	168.4 ±0.3	168.2 ±0.2	165.6 ±1.3
	K^+	3.38 ±0.15	3.47 ±0.13	3.85 ±0.03	4.06 ±0.03	4.15 ±0.05	4.23 ±0.10	4.00 ±0.09	4.04 ±0.04	3.90 ±0.11	3.80 ±0.08	3.80 ±0.10	3.85 ±0.09
ALDO-STERONE	Na^+	146.2 ±0.9	161.2 ±2.7	162.6 ±3.0	165.8 ±2.1	170.0 ±3.4	169.6 ±2.2	174.2 ±2.9	172.6 ±3.0	169.0 ±2.8	169.4 ±2.6	165.6 ±1.9	163.8 ±2.2
	K^+	3.45 ±0.05	3.39 ±0.12	3.68 ±0.18	3.90 ±0.09	3.98 ±0.08	4.02 ±0.05	4.04 ±0.08	4.10 ±0.10	4.00 ±0.14	3.96 ±0.09	3.85 ±0.10	3.87 ±0.13
ACTH	Na^+	145.5 ±0.4	162.8 ±1.5	165.8 ±1.8	166.0 ±1.5	168.0 ±2.0	167.0 ±2.4	165.0 ±2.2	165.6 ±2.4	166.0 ±1.5	167.0 ±1.9	167.0 ±1.6	166.4 ±1.3
	K^+	3.68 ±0.04	3.78 ±0.04	4.10 ±0.09	4.25 ±0.07	4.33 ±0.06	4.03 ±0.06	4.23 ±0.11	4.23 ±0.11	4.12 ±0.10	4.01 ±0.12	3.99 ±0.12	3.86 ±0.05
INTACT CONTROL	Na^+	144.1 ±0.9	158.4 ±0.7	163.5 ±2.1	166.5 ±2.5	167.5 ±2.7	168.9 ±2.7	169.3 ±2.9	167.5 ±3.0	167.1 ±2.4	168.1 ±2.1	166.4 ±2.3	165.6 ±2.1
	K^+	3.43 ±0.07	3.42 ±0.08	3.71 ±0.06	3.92 ±0.07	4.02 ±0.06	4.18 ±0.06	4.17 ±0.06	4.07 ±0.08	3.93 ±0.07	3.86 ±0.07	3.72 ±0.06	3.79 ±0.08
UNILATERAL ADX	Na^+	145.6 ±2.24	159.8 ±2.68	166.8 ±1.67	167.8 ±2.68	168.8 ±3.38	170.2 ±3.60	169.4 ±3.00	166.2 ±2.68	166.6 ±2.68	165.4 ±2.24	166.0 ±1.48	165.6 ±1.34
	K^+	3.79 ±0.17	3.51 ±0.04	3.75 ±0.08	3.85 ±0.13	4.04 ±0.12	4.07 ±0.15	4.10 ±0.13	4.04 ±0.12	4.01 ±0.13	3.86 ±0.06	3.72 ±0.06	3.65 ±0.04
TOTAL ADX	Na^+	138.0 ±1.73	155.8 ±2.14	157.4 ±1.61	162.0 ±1.48	164.6 ±1.26	167.8 ±1.95	168.2 ±2.14	167.8 ±2.68	169.2 ±2.79	169.2 ±3.00	168.4 ±2.79	167.2 ±2.68
	K^+	3.46 ±0.10	3.21 ±0.08	3.50 ±0.08	3.54 ±0.10	3.71 ±0.09	3.67 ±0.10	3.68 ±0.11	3.66 ±0.10	3.92 ±0.19	3.97 ±0.14	3.91 ±0.04	3.75 ±0.04

extra-renal activity in all but one bird. This bird showed a transient secretion 270 minutes after loading. At autopsy an adrenocortical remnant of approximately 5 mg. was found. If the results from these adrenalectomized birds were considered in the most unfavourable manner with respect to the control birds then it could be stated that 1 out of 5 adrenalectomized birds showed a measurable nasal secretion whereas 10 out of 10 control birds showed measurable nasal secretions.

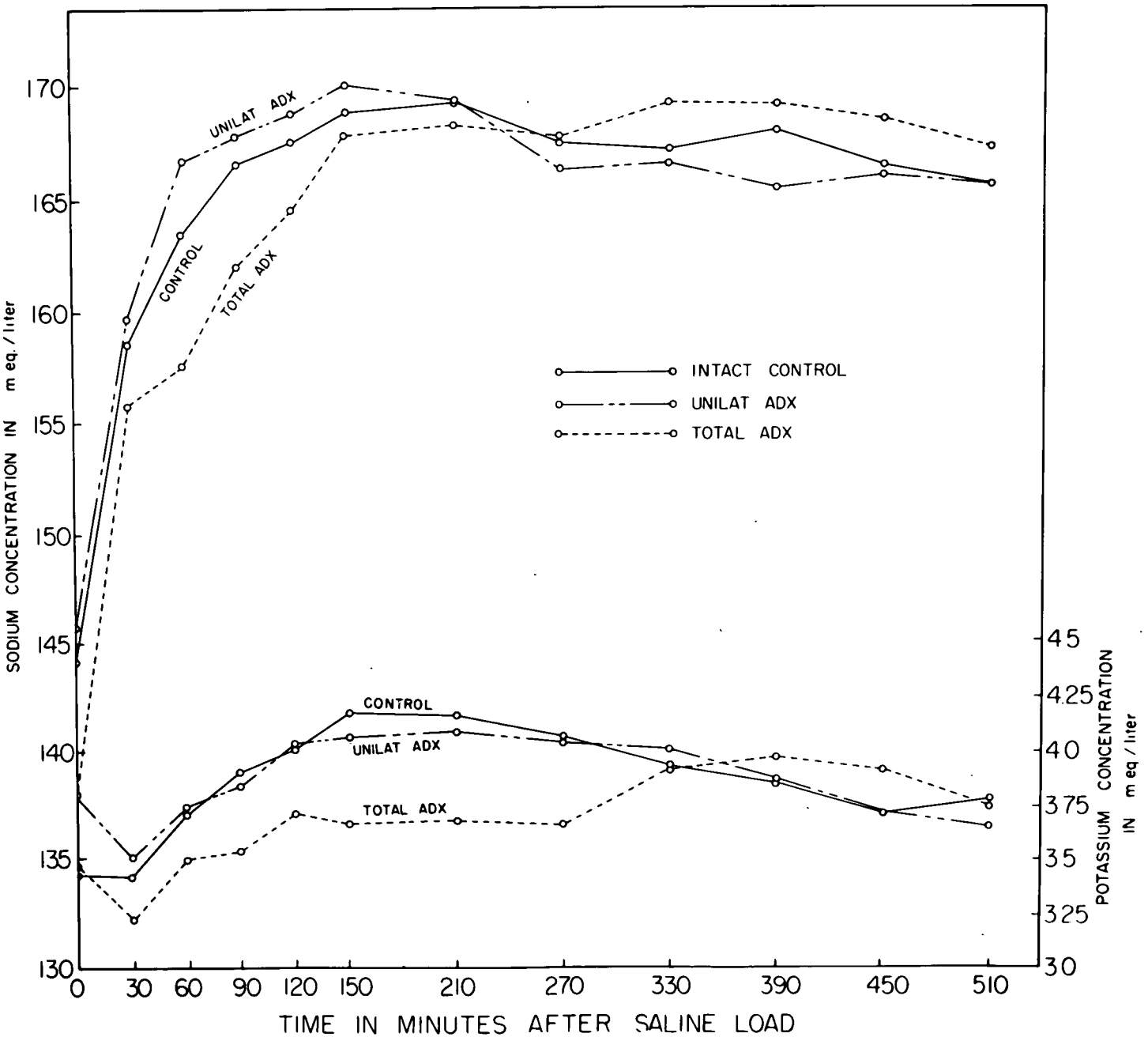
III. Serum Electrolyte Changes Tables II and V, Figures 7 and 8

The pre-loading serum sodium and potassium levels in the control, intact plus adrenocortical steroid and unilaterally adrenalectomized animals were normal although birds which had been totally adrenalectomized for nine to twelve hours before loading had significantly lower values. The mean serum sodium value was 114.1 ± 0.9 m.eq./liter for all experimental birds prior to saline loading with the exception of the totally adrenalectomized birds (138.0 ± 1.7 m.eq./liter).

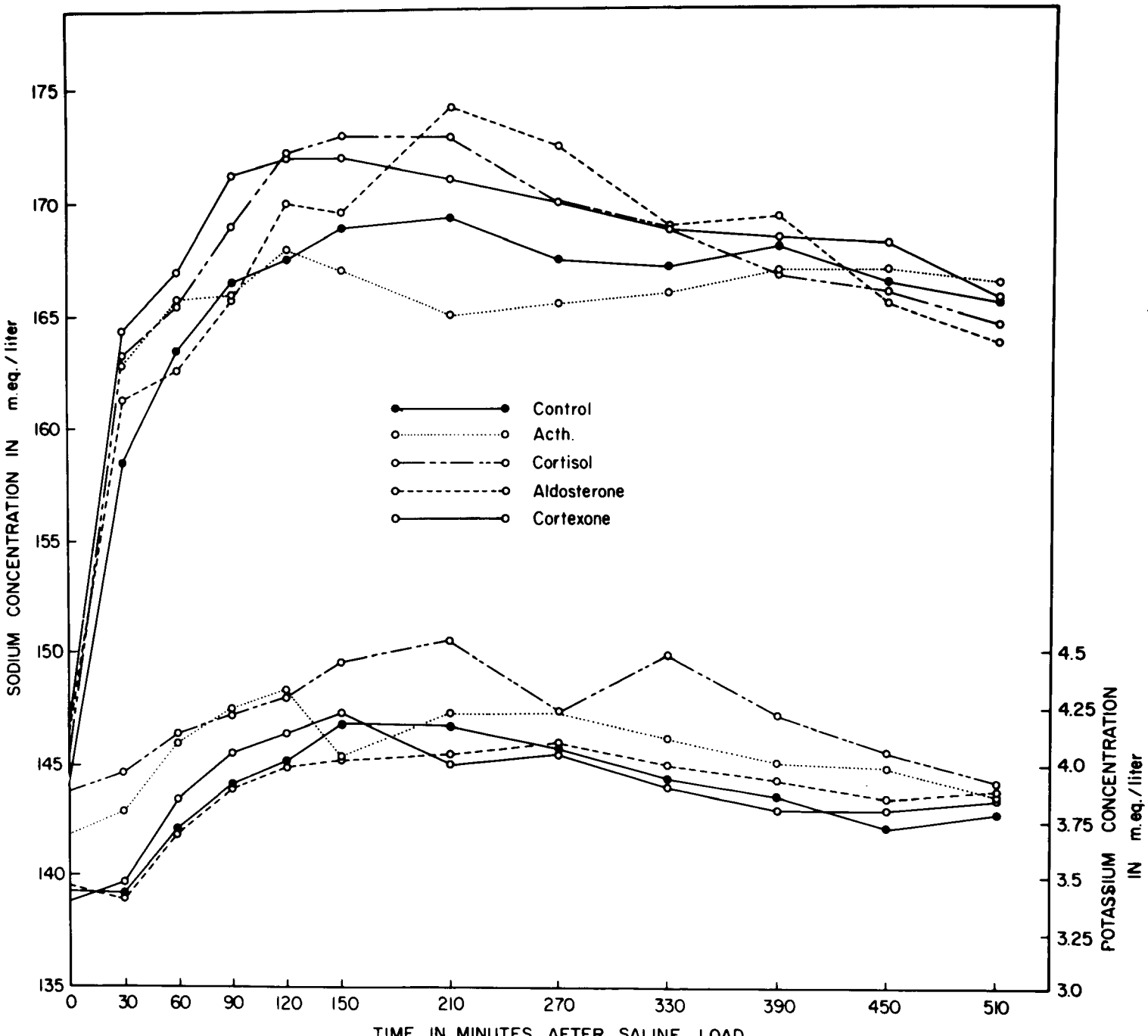
During the first hour after saline loading there was a steady rise in the serum sodium concentration of all groups of animals with the exception of totally adrenalectomized birds which lagged in attaining maximum levels. The serum sodium levels reached a plateau 90 minutes subsequent to saline loading.

Observation of the serum sodium levels at the onset of nasal secretion showed that activation of the nasal gland had occurred earlier in the birds treated with cortisol, cortexone, aldosterone and ACTH. The serum sodium concentration at the time of onset of nasal secretion in the Aldosterone and ACTH treated birds was 152.0 ± 1.2 and 151.8 ± 1.6 m.eq./liter respectively which was significantly lower than 160.6 ± 2.5 m.eq./liter in the control birds. The rise in serum sodium in the subtotally and totally adrenalectomized birds was slower and the nasal secretion occurred when the level reached 170.0 ± 3.4 and 176.0 m.eq./liter respectively. Treatment of totally adrenalectomized birds with cortisol caused nasal secretion at a serum sodium level of 155.7 ± 3.4 which was significantly lower than the control

Legend for Figure 7. The changes in serum levels of sodium and potassium in intact, totally and subtotally adrenalectomized ducks after hypertonic saline loading.


SODIUM CONCENTRATION IN m eq./liter
POTASSIUM CONCENTRATION IN m eq / liter
TIME IN MINUTES AFTER SALINE LOAD
INTACT CONTROL
UNILAT ADX
TOTAL ADX
CONTROL
UNILAT ADX
TOTAL ADX
CONTROL
UNILAT ADX
TOTAL ADX
170
165
160
155
150
145
140
135
130
45
4.25
40
3.75
35
3.25
30
0
30
60
90
120
150
210
270
330
390
450
510

Legend for Figure 8. The changes in plasma levels of sodium and potassium of adrenocortical steroid treated ducks after hypertonic saline loading.


SODIUM CONCENTRATION IN m.eq./liter
POTASSIUM CONCENTRATION IN m.eq./liter
TIME IN MINUTES AFTER SALINE LOAD
Control
Acth.
Cortisol
Aldosterone
Cortexone
175
170
165
160
155
150
145
140
135
4.5
4.25
4.0
3.75
3.5
3.25
3.0
0
30
60
90
120
150
210
270
330
390
450
510

level of 160.6 ± 2.5 m.eq./liter ($p < .001$).

IV. Adrenal weight changes

Treatment with 120 I.U. of ACTH during the five days prior to experimentation induced a 37% increase in the adrenal/body weight ratio. There was also a significant increase in the absolute adrenal weights from a control value of 157.4 ± 7.1 mg to 204.9 ± 12.1 mg after ACTH treatment ($p < .05$).

Discussion

The initial diuresis followed by a period of extra-renal sodium excretion in response to a saline load has been reported by Scothorne (15) for the Aylesbury variety of the domestic duck. Schmidt-Nielsen has also observed a similar diphasic response in the cormorant (Phalacrocorax auritus) after the oral administration of sea water (16).

A clue to the controlling factors associated with the extra-renal excretory mechanism in these birds was indicated by the renal electrolyte excretion pattern of the control group. There was a progressive decline in the renal sodium output coincident with the onset of nasal secretion. As well the concentrations of sodium and potassium in successive urine samples were compatible with an increased adrenocortical activity. Furthermore, in the birds treated with exogenous adrenocortical steroids and ACTH there was a direct correlation between the effectiveness of these hormones on the renal excretory pattern and the nasal secretory activity which ensued.

It may be suggested that the enhanced extra-renal secretion was a compensatory mechanism to offset the reduced renal excretion under adrenocortical influence. This, however, was clearly not the case with cortexone where the effect on renal sodium was not apparent in terms of total output. Nevertheless there was a significant increase in the nasal secretion. Previous workers (12) have suggested that the triggering mechanism associated with the onset of nasal secretion is of an osmoregulatory nature and it has been shown that the infusion of hypertonic sucrose will elicit a nasal response. The present work also suggested that some such osmoregulatory stimulus was necessary inasmuch as the onset of nasal secretion was always preceeded by a rise in serum sodium. This initial rate of increase in serum sodium was identical in all experimental and control groups but in the case of aldosterone and ACTH the onset of nasal secretion appeared independent of the

presumed osmoregulatory stimulus since it commenced when the sodium level was significantly lower than in the control animals. Certain preliminary studies substantiated this observation since it was observed that a spontaneous nasal secretion could be induced in normal unloaded ducks which had received intramuscular doses of cortisol, 9 fluorocortisol and aldosterone. This phenomenon was not observed with either cortexone or ACTH. It is interesting in this respect to note that Frings (17) working with the albatross observed that "...excitement or stress caused the nasal gland to become active when ordinarily it would not be".

The results obtained from the ACTH treated ducks were somewhat disappointing for although there was a 37% increase in total adrenal gland weight, and there was no reason to suspect that this was a medullary response, the increase in total nasal secretion was the least significant recorded. It is pertinent to note, however, that the endogenous adrenocortical activity in these birds brought about the highest initial response in terms of the nasal secretory rate. The relatively large doses of ACTH used may have exhausted or reduced the potential of the adrenocortical tissue to respond to the osmotic stimulus.

Further effects of the adrenocortical steroids on the extra-renal response included a three-fold increase in the initial rate of secretion and this rate was sustained in some cases (cf. cortisol and aldosterone) for a longer period than in the control ducks. This resulted in the increased extra-renal loss of sodium although the concentration of the nasal secretion in none of the hormone treated groups was as high as in the controls. It was, however, significantly lower in the case of cortisol and cortexone treated birds. This suggested the possibility of some other factor or factors involved in the extra-renal response.

It was also apparent that bilateral adrenalectomy in the duck completely abolished the ability of the nasal gland to respond to a saline load. This ability

could be restored in the adrenalectomised duck by the administration of cortisol. It is clear, therefore, that one essential controlling factor for nasal secretion, namely the adrenocorticosteroids, must be added to those involving the nervous system (12,18).

Birds which normally manifest the phenomenon of extra-renal secretion in response to a salt load can be made to secrete from the nasal glands by such other methods, as increasing the blood osmolarity through intravenous infusion of sucrose (15) or by the injection of certain adrenocorticosteroids without the addition of hypertonic fluids (19). The secretion achieved by the latter means was, however, only transient and it was concluded that the full expression of the response is dependent on both increased osmolarity of the blood together with elevated values of circulating adrenocorticosteroids. This introduces the concept that before the nasal gland will function fully there are required threshold values which must be exceeded not only for the degree of hypertonicity of the blood but also for the circulating amounts of corticosteroids. Moreover, it is clear that nasal secretion does not occur in the adrenalectomized duck, that is in the absence of adrenocorticosteroids, however elevated might be the osmolarity of the extra-cellular fluid. Cortisol administration following adrenalectomy effectively re-establishes the extra-renal mechanism. Thus, the adrenocorticosteroids have a direct and essential action on the extra-renal salt glands. Further, the delay in onset, duration and magnitude of the extra-renal response was proportional to the level of circulating adrenocorticosteroids, as unilaterally adrenalectomised ducks, exhibiting relative adrenocortical insufficiency, had a delayed extra-renal response which occurred at a higher blood osmolarity than that seen for the control group of ducks. Conversely, simulated hyperadrenocorticalism (19) accelerated the onset of the extra-renal response but at a lower value for blood osmolarity. These contrasting conditions implied that a reciprocity exists between the concentration of circulating

corticosteroids and the osmolarity of the extra-cellular fluid. This relationship was well illustrated in the one adrenalectomised animal which was found at autopsy to possess a small adrenal remnant, thus manifesting severe adrenal insufficiency. In this animal, a transient secretion from the nasal glands occurred after a very long delay and with the highest concentration of serum sodium recorded in these and previous studies (19).

The day-to-day fluctuations in electrolyte intake in laboratory ducks and in marine birds on a similar diet with freshwater to drink are well within the capacity of the kidney alone to regulate the electrolyte and fluid distribution between the various compartments of the body (20). Under conditions of saline loading in the duck and in marine birds after the ingestion of seawater, there was a dual and diphasic response (15, 19) the renal phase preceding and of shorter duration than the extra-renal phase. Thus, in the normal physiology of a marine bird it would follow that the nasal gland only functions fully under conditions of hyperosmolarity with attendant hypersecretion of adrenocorticosteroids and under these conditions alone the normal capacity of the kidney to regulate is exceeded. It can be supposed, therefore, that the experimental addition of sodium chloride to ducks mimics a normal dilemma of the marine bird and brings into play the capacity to secrete, through the nasal glands, a saline solution which is three to four times more concentrated than the extra-cellular fluid, thereby maintaining homeostasis by reversing the trend to accumulate sodium and lose water from the body. Hence the change from renal to extra-renal routes of excretion in the saline loaded duck was a dynamic shift to a more economical method of restoring homeostasis.

The ducks investigated here showed a negative water balance in the initial period after the administration of a saline load but later urine formation diminished and then ceased. Thus, the renal phase waned as the extra-renal phase became dominant. Whilst undoubtedly the initial water loss may rest on the osmotic demands of the excretion of excess sodium both in control and adrenalectomised animals it

would appear likely that hormones intervene in the determination of the pattern of the renal phase. One such hormonal factor may well be antidiuretic hormone (ADH) and this is known to occur in birds (21). In mammals saline administration evokes a secretion of ADH (22) and there is a relationship between the activities of the adrenocortical hormones and antidiuretic hormone at the kidney level (23, 24). It can, therefore, be argued that saline loading in control and adrenalectomized ducks and in adrenalectomized ducks receiving cortisol, resulted in an increase in ADH secretion with consequent water reabsorption and cessation of urine formation. Concomitantly, increasing amounts of sodium were reabsorbed with an increasing rate of adrenocorticosteroid secretion in control ducks and the injection of cortisol into adrenalectomized ducks mimiced this condition. Thus, the pattern of renal excretion of salt and water in the control and cortisol injected adrenalectomized ducks was consistent with adrenocortical hypersecretion combined with enhanced ADH secretion since there was a gradual fall with time in sodium output, urine sodium concentration and volume of urine produced. On the other hand, in the adrenalectomized ducks there was a marked polyuria with enhanced sodium loss. However, the diminished glomerular filtration rate in the absence of ACH, together with the possible increasing secretion of ADH consequent to an elevated blood osmolarity, may have caused the reabsorption of water and hence the termination of urine formation; although the time during which urination occurred was greatly protracted compared with control animals. Cortisol treatment of the adrenalectomized ducks given a saline load, restored the renal phase to normal presumptively by increasing the glomerular filtration rate, by its positive action as a diuretic agent, by enhancing the retention of sodium in the kidney tubule and by restoring the secretory capacity of the extra-renal salt glands.

It might be argued that the differences between the normal and adrenalectomized ducks in their handling of a saline load by renal and extra-renal mechanisms can

be accounted for, on the one hand, by increased corticosteroid secretion causing enhanced renal sodium retention and thereby elevating serum sodium values, whilst on the other, the absence of adrenocorticosteroids in the adrenalectomized animal resulted in renal sodium loss and a depression of serum sodium values. However, observations revealing equivalent serum sodium values in both normal and adrenalectomized saline loaded animals, despite the significantly lower values in the latter previous to loading, vitiated this approach and emphasizes the essential role of the adrenocorticosteroids in determining the pattern of the diphasic response.

The predominant adrenocortical secretions found in birds are corticosterone and aldosterone (25, 26, 27). If the secretory pattern is similar in the duck, then on general grounds, saline loading would be expected to inhibit aldosterone secretion as it does in the mammal (28). The nasal gland, however, will only respond fully when elevated levels of adrenocorticosteroids are present and it is therefore presumed that the glucocorticoid component of the adrenocortical secretion is responsible. The efficacy of cortisol in restoring the extra-renal phase to the adrenalectomized saline loaded duck substantiates this conclusion. Further, cortisol but not aldosterone was effective in causing nasal secretion to occur in the normal duck in the absence of a hypertonic saline load (20). The factor responsible for the elevated levels of adrenocorticosteroids may well be ACTH (19) and it is interesting in this connection that other workers (27) have found that the injection of ACTH into various gallinaceous birds significantly enhances the corticosterone levels found in the adrenal venous effluent.

It is relevant to consider that if in the saline loaded duck, and also in the marine bird, ingestion of hypertonic fluids lead to an enhanced secretion of ADH, then this factor may well evoke an enhanced secretion of adrenocorticosteroids. It has been shown in mammals that saline loading stimulates adrenocortical activity

(29) and the posterior lobe hormones are clearly involved (30,31). On this analogy we can say that ADH secretion either brings about an obligatory release of ACTH (29) or that ADH stimulates directly the biosynthetic pathways of adrenocorticosteroidogenesis (31). In the duck then, one or both of these mechanisms may have operated to effect an enhanced secretion of ACH, thereby overcoming a threshold value to cause extra-renal excretion of a saline hypertonic to extra-cellular fluids. The combined effect of enhanced ADH and ACH secretion suppressed the kidney so that urine formation ceased. The extra-renal phase, however, persisted under the influence of ACH until either the osmotic "stress" is removed or the circulating adrenocorticosteroids fall below the threshold level. In the marine bird it is envisaged that the following sequence of events occur:- ingestion of sea water, elevation of the sodium chloride content of the blood, rise in the level of circulating adrenocorticosteroids, nasal gland secretion, reduction of the sodium chloride content of the blood, fall in the level of circulating adrenocorticosteroids and cessation of nasal gland secretion.

In the present experiments when saline was given acutely, nasal gland secretion waned and in some cases terminated despite high osmolarity of the blood. There may, then, have been excessive stimulation of adrenal tissue to a point where the enhanced biosynthesis of adrenocorticosteroids could no longer be maintained. It is known, for example, that adrenocortical exhaustion can be produced by excessive ACTH stimulation in the Capon (25). Such a suggestion is supported by the observations on unilaterally adrenalectomized ducks, when the decrease in adrenocortical tissue and secretion is reflected by a marked shortening of the period during which extra-renal secretion occurs.

With the possible exception of the Amphibia, the periodic exposure to a marine environment is found in all vertebrate classes. The anadromous teleosts, for example, are able to withstand wide variations in the tonicity of their environment,

indeed, the salmon and the steelhead trout migrate from fresh water to sea water and back in the natural course of their lives. Just as in the case of the marine bird on a sea water diet, in order to survive this transfer from a hypotonic to a hypertonic environment, the fish must excrete excess quantities of sodium and potassium without the loss of commensurate volumes of water. Comparison of the cummulative output of sodium from saline loaded trout in the presence and absence of certain adrenocortical steroids (4) with the cummulative output of sodium from similarly treated ducks showed that, although the quantities of sodium and the time relationships are not strictly comparable, the same basic pattern of excretion was observed in response to these steroids.

Consideration of the problems which confront an animal exposed to a hypertonic sea water environment or to a long term diet containing sea water indicate that the animal must conserve water and simultaneously excrete sodium, potassium and chloride which are the major ionic constituents of sea water. The secretion of a nasal discharge containing sodium at a level higher than 100% sea water (600 V 470 m.eq./liter) and with an Na/K ratio well within the limits of sea water (36.0 ± 3.9 V 48.3) is clearly a mechanism whereby sodium and potassium may be lost without the excessive excretion of water.

Even though the present experiments were of a chronic nature and the total volumes excreted by the birds were greater than the intake, there was probably some antidiuretic factor operating, since the renal phase of excretion ended so abruptly during the first hour. Changes in the neuro-secretory activity of the hypothalamus have been observed in fish during the first 4 hours after transfer to sea water (32, 33). Carlson (34) has observed changes in the amounts of oxytocic and vasopressin-like substances in the neurohypophysis of the steelhead trout (Salmo gairdnerii) after transfer to 60% sea water.

In summary, it is suggested that adrenocortical steroids of the glucocorticoid

type are an essential concomitant for the functioning of the extra-renal salt glands in the duck and with antidiuretic hormone determine the characteristic diphasic response which follows ingestion of hypertonic saline loads. These hormonal mechanisms together effectively restore homeostasis by causing a dynamic shift from the renal route to the physiologically more economical extra-renal route of excretion. The survival value of these mechanisms is self-evident and is especially advantageous to birds who, by virtue of their ecological niche, are subjected to unusually high intakes of seawater or marine invertebrates.

The exact locus of action of the adrenal hormones on the nasal gland is not known and remains the subject for future investigations. The recent report of Hokins (35) would, however, suggest an involvement in some stage of the phosphatidic acid cycle.

CONCLUSION

It has been shown in this investigation that:-

(a) the intact saline loaded duck (Anas platyrhynchus) exhibits a diphasic excretory pattern as an initial diuresis followed by activation of the supra-orbital glands.

(b) the adrenocortical steroids administered to an intact saline loaded duck vary both phases of this response.

(c) total adrenalectomy decreases the extra-renal response of the duck to hypertonic saline loading whereas total adrenalectomy completely abolishes this response.

(d) the administration of hydrocortisone to the totally adrenalectomized duck causes complete reactivation of the supra-orbital gland - suggesting the essential role of a glucocorticoid in its function.

Thus it is evident that the supra-orbital gland in the Domestic duck is a site for the extra-renal excretion of electrolytes and that it is an efficient system whereby certain marine birds may excrete dietary salt loads while maintaining constant the "milieu interieur".

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