THE EFFECTS OF EXOGENOUS ALDOSTERONE AND CORTICOSTERONE ON RENAL FUNCTION IN THE ADULT MALE DOMESTIC CHICKEN (GALLUS DOMESTICUS)

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ABSTRACT

THE EFFECTS OF EXOGENOUS ALDOSTERONE AND CORTICOSTERONE ON RENAL FUNCTION IN THE ADULT MALE DOMESTIC CHICKEN (Gallus domesticus)

By

Edward A. Cogger

Two experiments were conducted to determine the effects of exogenous administration of the two primary avian adrenocortical steroids on renal function in the adult male chicken. The steroids administered were corticosterone (a glucocorticoid) and aldosterone (a mineralocorticoid). The method, duration, and quantity of administration were varied in the two experiments. Urine was collected in these experiments from anesthetized (sodium phenobarbital) roosters by a technique which surgically modifies the cloaca to separate urine and feces, and is only useful for acute collections.

In the first experiment, corticosterone (3.0, 6.0, 9.0, and 12.0 μg/min) and aldosterone (0.04, 0.08, 0.12, and 0.16 μg/min) were infused for 95 minutes and during this time plasma concentration of sodium (P_{Na}) and potassium (PK), glomerular filtration rate (GFR), urine flow rate (V), and excretion rates of sodium (E_{Na}) and potassium (E_K) in the liquid phase of the urine were monitored in nine 10 minute periods. P_{Na}

remained stable for the duration of the experiment. Though P_K showed greater variability than P_{Na}, this was not related to the steroid treatments. GFR was not affected by aldosterone but there was a significant ($p < .05$) interaction between level and time when corticosterone was infused. At 6.0 and 9.0 μg/min GFR increased by 0.44 ($p < .10$) and 0.78 ($p < .05$) ml/min/kg, respectively, after 55-75 minutes of infusion but waned during the 75-95 minute period. The aldosterone treatments had no effect on V or percent water reabsorbed (Reab $_{H_2O}$) but there was an experiment-wide time effect ($p < .05$). V remained stable up to 50 minutes (23.5 μl/min/kg) during infusion then began to increase until the end of the experiment (28.4 μl/min/kg). This was accompanied by a decrease in Reab $_{H_2O}$ (98.34% to 97.90%). Corticosterone at 9.0 and 12.0 g/min caused significant ($p < .05$) increases in V by 55-75 minutes and 30-50 minutes, respectively. The diuresis at the 9.0 μg/min treatment (from 18.4 to 41.9 μl/min/kg) was the result of the change in GFR exhibited by this group whereas the diuresis seen in the 12.0 g/min treatment (from 29.1 to 81.4 μl/min/kg) was related to a decrease in Reab $_{H_2O}$.

Corticosterone at 3.0, 6.0, and 9.0 μg/min caused significant ($p < .05$) increases in E_{Na} by 55-75 minutes (.99, .75, and .87 μeq/min/kg) which declined during the 75-95 minute period. When the excretion rate was expressed as a percent of

the filtered load of sodium only the 3.0 μg/min treatment showed a significant ($p < .05$) increase in excretion (from 0.33 to 0.81% by 55.75 minutes). There was no effect by any treatment level on the E_K which did rise (from 0.81 to 1.04 μeq/min/kg) significantly ($p < .05$) on an experiment-wide basis.

Aldosterone at the doses given did not affect the E_{Na} during the 95 minutes of infusion but E_K was significantly affected ($p < .05$). Aldosterone at 0.08 and 0.12 μg/min exhibited contrasting actions. The former increased E_K by .42 μeq/min/kg whereas the latter decreased E_K by .48 μeq/min/kg at 55-75 minutes. E_K was returning toward initial values when the experiment was terminated. The clearance of potassium (C_K) followed the same trend at 0.08 μg/min, however at 0.12 μg/min it remained depressed until the end of the experiment. When expressed as percent of filtered load no significant effects on potassium excretion were observed.

In the second experiment, the male chickens received either 14.0 mg/kg/day of corticosterone in stabilized suspension (0.9% saline with 1% methyl cellulose), 0.35 mg/kg/day of aldosterone in safflower oil, or one of the carrier substances. At the end of three days of treatment urine was collected for a 30 minute period. Sodium and potassium excretion rates were measured in the liquid and solid phases of the urine. A significant ($p < .01$) diuresis was observed in the

corticosterone treated birds (76.2 in contrast to 12.4 µl/min/kg). This was accompanied by a significant decrease in water resorption ($p < .01$) and body weight ($p < .05$).

Aldosterone and corticosterone significantly depressed the sodium excretion rate in liquid, solid and total urine. The total excretory rates of sodium were 82.3 ± 24.4 (SD), 36.5 ± 3.6, and 27.1 ± 9.0 µeq/30 min/kg for control, corticosterone, and aldosterone treated birds, respectively. Corticosterone caused a significant ($p < .01$) increase in the excretion rate of potassium in liquid and total urine while aldosterone had no effect. The excretion rate of potassium in the solid urine was unaffected by the steroids. The potassium excretory rates in total urine were 40.6 ± 17.5 (SD), 103.9 ± 27.1, and 36.5 ± 13.3 µeq/30 min/kg for control, corticosterone, and aldosterone treated birds, respectively.

The percent of sodium and potassium excreted in solid urine was significantly ($p < .01$) reduced by corticosterone (sodium, 15.2% versus 3.7%; potassium, 39.2% versus 11.1%). This lowered percentwise excretion of the sodium and potassium may be indicative of increased excretion of hydrogen or ammonium ions, which have a higher affinity for urate.

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INTRODUCTION

The avian kidney is an intriguing organ anatomically and physiologically. It has three types of nephrons: those with loops of Henle (like mammalian nephrons), those with short loops which are anatomically different from the loops of Henle, and those with no loops of Henle (like the reptilian nephron). These are arranged in the multilobar organ in many separate medullary, and their associated cortical, lobules. In addition to the arterial blood supply, there is a functional venous portal system routing blood to the peritubular capillaries. The renal portal valve is strategically located so it can control the flow of blood into the portal system.

The kidney of birds has limited concentrating ability. The domestic chicken will excrete urine only twice as concentrated osmotically as plasma under dehydrating conditions; some other birds can excrete urines with four-five fold osmotic concentrations. However, the bird excretes its nitrogenous waste as uric acid rather than urea; the uric acid precipitates in the urine and becomes osmotically inactive. The uric acid precipitate is arranged in irregular layers trapping soluble matter

which contains significant quantities of sodium and potassium. This further reduces the osmolality of the liquid urine. Many birds which are adapted to marine environments possess functional salt secreting organs in addition to the kidney. Also, significant post-renal modifications of the urine occur in the cloaca, colon, and/or ceca of birds.

Renal studies of electrolyte and water excretion in birds have been conducted primarily with species adapted to marine and arid environments. Most studies have involved salt loading, water loading, and/or dehydration. In general the role of the adrenal steroid hormones in salt and water metabolism has been little studied in birds and less so in the domestic chicken. The research reported herein was undertaken to study the role of the primary avian adrenal steroids on renal function in the domestic chicken.

OBJECTIVES

1. To ascertain the effects of the primary avian adrenal steroid hormones, aldosterone and corticosterone, on renal function in the adult male chicken.

2. To determine if the excretion of sodium and potassium in liquid urine is indicative of their excretion in total urine.

3. To develop a technique for acute collection of urine in the adult male chicken.

REVIEW OF LITERATURE

The Urinary Apparatus

Gross structure

The avian kidneys are paired organs which lie ventrolateral to the vertebral column within the boney depression of the pelvis. They are relatively large organs ranging from 1.0 to 2.6 (Benoit, 1950 as cited by Sturkie, 1965) and 0.6 to 2.1 (Johnson, 1968) percent of the body weight. Johnson (1968) observed an inverse relationship between the relative kidney size and body weight which was attributed to metabolic needs. No sex difference was observed. In the chicken (Gallus domesticus) the kidney is divided into anterior, middle, and posterior divisions (Goodchild, 1956 as cited by Siller and Hindle, 1969) which are not related to the internal morphology (Siller and Hindle, 1969). The ureters transport the urine from the kidney to the urodeum of the cloaca. In the chicken, no bladder is present.

The nephrons and their organization

The avian kidney is a multilobar organ, but there is disagreement in the literature as to what constitutes a lobe. Since the avian ureteral-collecting duct complex is a continuous

dendritic system, analogies to the mammalian renal pelvis are difficult to make. Johnson and Mugaas (1970b) considered a lobe to be one medullary lobule and its peripheral cortical lobules, whereas Siller and Hindle (1969) considered it to be the complex of medullary lobules and their associated cortical lobules drained by a single uretral branch. In the chicken, Siller and Hindle (1969) found a single cortical lobule for each medullary lobule; this was rare among other species (Johnson and Mugaas, 1970b).

The cortical lobule is bounded by the afferent interlobular veins of the renal portal system (see below) and has a central efferent intralobular vein with the proximal convoluted tubules central to the glomeruli, and distal convoluted tubules and collecting ducts peripheral to the glomeruli (Spanner, 1925; Siller and Hindle, 1969; Siller, 1971). The medullary lobule is an aggregation of the thick and thin segments of the loops of Henle from the juxtamedullary nephrons, the collecting ducts, and a vasa recta (Spanner, 1925; Sperber, 1948; Siller and Hindle, 1969; Johnson and Mugaas, 1970b; Siller, 1971). Poulson (1965) demonstrated a relationship between the numbers of loops of Henle and the ability to concentrate urine in some passerines. This finding was supported by Johnson and Mugaas (1970a) and extended to implicate length of the loops of Henle and interlobular association of the medullae. These observations suggest a countercurrent multiplier system in passerines which

has been demonstrated in chickens and turkeys (Skadhauge and Schmidt-Nielsen, 1967b).

Huber (1917) described three types of nephrons in the avian kidney and a comprehensive description of their structure was presented by Siller (1971). The most abundant type is the cortical (reptilian) nephron which consists of a glomerulus, a proximal and distral convoluted tubule, and a collecting duct. The juxtamedullary (mammalian) nephron also contains a loop of Henle which extends into the medullary lobule. The third is an intermediate type with a short medullary loop unlike the loop of Henle.

The glomeruli in birds are smaller and simpler than mammalian glomeruli. They consist of a few (sometimes only one) capillary loops surrounding a mass of mesangial cells (Siller and Hindle, 1969). The ultrastructure of the avian glomerulus compares to that of mammals with the exception of a central mass of mesangial cells (Siller, 1971). A juxtaglomerular apparatus has been described by several authors (Edwards, 1940; Smith, 1966; Sokabe et al., 1969; Capelli et al., 1970; Siller, 1971).

The blood supply

There are three renal arteries to each kidney of the domestic chicken. The anterior renal artery originates on the aorta and supplies blood to the adrenal, testes or ovary, and the

anterior division of the kidney. The middle and posterior renal arteries arise together from the sciatic artery and supply blood to the middle and posterior division of the kidney (Siller and Hindle, 1969). Sperber (1948) reported similar findings but also stated that one or more fine branches to the kidney originate from the femoral artery. Siller and Hindle (1969) were unable to confirm this finding. As the renal arteries ramify the renal tissue their branches appear to follow the efferent veins; and the intralobular arteries to each lobule originate from one or more of these branches (Sperber, 1948; Siller and Hindle, 1969). The intralobular arteries are arranged symmetrically around the central vein of the lobule. The intralobular artery gives off afferent arterioles that are directed toward the periphery of the lobule and break up into the capillaries of the glomeruli. The efferent arteriole is also directed toward the periphery and enters the peritubular capillaries against the flow of the renal portal blood (Sperber, 1948; Siller and Hindle, 1969).

The kidney of birds has a renal portal system. The renal portal system supplies venous blood to the anterior, middle, and posterior divisions of the kidney and its capability of functioning has been demonstrated (Sperber, 1946, 1948, 1960). The caudal and cranial renal portal veins originate on the external iliac vein (femoral vein) between the point where it enters the body cavity and the renal portal valve. They supply the middle

and posterior lobes, and anterior lobe, respectively (Sperber, 1948; Akester, 1964). The cranial renal portal vein was shown to be continuous with the vertebral venous sinuses (Akester, 1967a) and to give rise to the interlobular veins of the kidney's anterior division (Sperber, 1948). The caudal renal portal vein courses caudally through the renal tissue. The sciatic vein joins it between the middle and posterior divisions of the kidney. The caudal renal portal veins from an anastomosis with the hypogastric veins from the tail region and the coccygeomesenteric vein (Sperber, 1948; Akester, 1964). The caudal renal portal vein gives rise to the interlobular veins of the kidney's middle and posterior division (Sperber, 1948). The interlobular veins are continuous with the peritubular capillaries which are drained by the intralobular or central vein (Sperber, 1948; Akester, 1964; Siller and Hindle, 1969). The intralobular veins leave the lobule and eventually drain into the anterior and posterior renal veins. These veins form an anastomosis with the external iliac vein just downstream from the renal portal valve and the large vein formed unites with the vein from the contralateral side to form the posterior vena cava (Sperber, 1948; Akester, 1964). The location of the renal portal valve suggests that it can control the flow of venous blood to the kidney or away from the kidney to the heart. Physiological evidence for this possibility was suggested by Sperber (1948), Rennick and Gandia

(1954), and Akester (1964, 1967).

Methods of Collecting Avian Urine

The collection of avian urine is complicated by its mixing with the feces in the cloaca. A number of techniques for obtaining ureteral urine have been described in the literature and no single procedure seems applicable to all needs. These procedures fall into five general categories.

Two of those are best adapted to chronic studies, particularly nutritional experiments. The first is the colostomy described by Weiner (1902) and subsequently used by many investigators. This involves the formation of an artificial anus in the abdominal wall. Procedures for exteriorization of the ureteral openings have been described by Hester et al. (1940), Hart and Essex (1942), and Dixon and Wilkinson (1957). Though these differ in specific surgical details they all involve reconstruction of the cloaca such that the ureteral openings are moved dorsally to the base of the pygostyle. Both colostomy and exteriorization of the ureters involve considerable postoperative care to remain functional (Sperber, 1960) and in the case of colostomy intestinal tonus problems are evident (Hart and Essex, 1942). At least in one instance (Hart and Essex, 1942) after colostomy and exteriorization of the ureteral openings one percent NaCl had to be added to the diet to maintain viability of the birds. One modification of the Dixon and Wilkinson procedure was claimed to have

overcome the problems of cloacal fistulation and wound dehiscence (Elliott and Furneaux, 1971). Since the cloaca and rectum seem to be involved in final urine modification (to be discussed below), renal clearance studies after chronic use of these techniques is questionable (Sykes, 1971).

Techniques for acute collection of avian urine can be divided into three categories. The first is direct cannulation of the ureters (Sharpe, 1912) which is accompanied by a diuresis for at least thirty minutes (Hester et al., 1940). These cannulas tend to become clogged with urates. The second is a funnel technique which has many variations. Davis (1927) restrained the bird on its back with the head tilted upward and used a large catheter to drain the cloaca near the ureteral openings. The birds were starved and water loaded, but occasionally the rectum had to be plugged with cotton to prevent contamination. This was modified by Hart and Essex (1942) using a glass cannula with a glass ball which fit in the rectum, positioning the cannula opening under the ureteral openings allowing the bird to remain in the upright position. Another modification (Bokori, 1961 as cited by Sykes, 1971) used a dual cannula open to the rectum and the ureters. An initial diuresis normally occurs from the physical handling of the bird when using this type of technique (Hester et al., 1940; Hart and Essex, 1942).

The third technique was originally developed by

Sperber (1946, 1948) to investigate the existence of a functional renal portal system in the chicken. He simply sutured a small funnel which was fitted with a separate larger collar over the ureteral openings. Because of the highly viscous nature of avian urine, this was subsequently modified by perfusing the funnels with a rinsing solution to prevent clogging (Lindahl and Sperber, 1956; Campbell, 1960). This method has been widely used particularly where collection from the separate kidneys is required.

Avian Renal Function

The elements of renal function

Cuypers (1959) demonstrated that glomerular filtration is essential to urine formation in the chicken. He showed that urine flow ceased after interruption of the arterial blood supply to the kidney even though the renal portal system was intact. The glomerular filtration rate (GFR) is central to the study of renal function. Evidence suggests that the clearance of inulin, a fructose polysaccharide, meets the criteria for measuring GFR, i.e., it is (1) freely filterable through the glomerular capillary membrane, (2) biologically inert and neither reabsorbed nor secreted by the nephron, (3) nontoxic and does not alter renal function, and (4) quantifiable in plasma and urine (Pitts, 1963). The ratio of inulin clearance to glucose clearance in the chicken is equal to 1.01 after phlorizin

treatment (Pitts, 1938). GFR has been reported by various authors to range between approximately one to three milliliters per kilogram body weight per minute in the chicken (see Pitts, 1938; Korr, 1939; Sperber, 1960; Dantzler, 1966; Skadhauge, 1964; Skadhauge and Schmidt-Nielsen, 1967a). Large variations in GFR have been reported within and among chickens (Langford and Fallis, 1966). In the chick, GFR increases at hatching and attains adult levels at nine days of age (Cooke and Young, 1970). In other birds, the budgerygah (Krag and Skadhauge, 1972) and the duck (Holmes, 1965; Holmes et al., 1968) have GFR's of slightly less than four and more than two milliliters per kilogram per minute, respectively.

Tubular reabsorption occurs in the avian kidney. One of the most conspicuous examples of this is water. In the chicken as much as 99 percent of filtered water can be reabsorbed (Skadhauge and Schmidt-Nielsen, 1967a) whereas in the budgerygah, an inhabitant of arid areas, even greater reabsorption of water can occur (Krag and Skadhauge, 1972). Tubular reabsorption of a solute is said to have occurred when its clearance is less than the clearance of inulin (GFR). Pitts and Kerr (1938) and Korr (1939) studied the reabsorption of urea. Essentially no glucose is present in chicken urine and its reabsorption has been studied (Pitts, 1938; Sperber, 1960; Dantzler, 1966). Data from numerous sources indicate that sodium and/or potassium are

reabsorbed in the chicken (e. g. Skadhauge and Schmidt-Nielsen 1967a; Sykes, 1971) but no detailed micropuncture studies have been reported. An electrophysiological short circuit current study indicates that, at least for the pigeon, the intensity of active ion transport in the proximal and distal tubules is approximately 50% and 25%, respectively, of that in the rat and this transport is depressed similarly in the rat and pigeon by furosimide and strophanthin (Bessonov, 1972).

When the clearance of a solute exceeds the inulin clearance (GFR) tubular secretion has occurred. Since uric acid is the primary form of nitrogen excretion, its tubular secretion has been extensively investigated (Sykes, 1971). It has been shown that as much as 93% of excreted uric acid is contributed by tubular secretion (Shannon, 1938). Rennick et al. (1952), using the Sperber (1948) technique and radioactive labelled potassium, demonstrated that the potassium ion is secreted by the chicken's kidney. Orloff and Davidson (1956, 1959) have estimated the secretory transport maxima, and established competitive inhibition with the hydrogen ion and non-competitive inhibition with a mercurial diuretic. They suggest from these data and others (Orloff and Burg, 1960) that all potassium excreted is secreted in the distal tubule by a linked transfer of potassium, hydrogen and sodium ions similar to that suggested by Pitts (1958).

Renal response to osmotic challenge

Water loading was used by some of the early investigators of renal function in the chicken to increase urine flow (see David, 1927; Coulson and Hughes, 1930; Pitts, 1938). Pitts (1938) suggested that GFR and urine flow were independent except shortly after the bird had received a large dose of water; then both were elevated. After the administration of water a diuresis curve with a concommitant decrease in urine osmolarity has been observed (Korr, 1939; Dicker and Halsam, 1966; Skadhauge and Schmidt-Nielsen, 1967a; Sykes, 1971). Dicker and Halsam (1966) found two peaks in the diuresis. The first occurred within 20 minutes of water gavage which was thought to be neurogenic in origin as a response to distension of the crop since it could be reproduced by filling the crop with liquid paraffin. The second coincided with the release of water from the crop and absorption by the gut. Korr (1939) attributed the increased urine flow to an increase in the GFR from 1.1 to 2.5 ml per minute per kilogram and not a decrease in water reabsorption; as he stated, "on the contrary more water is reabsorbed at high urine flow than at low urine flow . . . ." Dicker and Halsam (1966) supported this thesis; they did not measure GFR but instead used endogenous creatinine excretion as an index of GFR. On the contrary Skadhauge and Schmidt-Nielsen (1967a) found only a slight increase in GFR and a large decrease in water reabsorption when

water loading a previously dehydrated rooster. The observed change in urine flow rates was comparable to those measured by Korr (1939). In all cases during the diuresis the urines became very hypotonic. In chickens and turkeys a decrease in the medullary-cortical osmotic gradient has been observed following water loading (Skadhauge and Schmidt-Nielsen, 1967b). Sodium excretion remains relatively unaffected during the diuresis whereas potassium excretion is slightly enhanced and chloride excretion is quadrupled (Sykes, 1971).

In the chicken, dehydration results in a decrease in urine flow and an increase in urine tonicity (Korr, 1939; Skadhauge and Schmidt-Nielsen, 1967a). Urine to plasma osmotic ratios can become as high as 2. Urine is normally isotonic or slightly hypotonic to plasma (Korr, 1939). Korr suggested that GFR decreases during dehydration to approximately 50 percent of the control value. This was not substantiated by Skadhauge and Schmidt-Nielsen (1967a) who found only a slight decrease in GFR as compared to water loaded birds. These authors suggest that the rooster can resorb more filtered water than mammals for a given urine to plasma osmotic ratio because uric acid takes up little osmotic space as compared to urea. They also found that more of the sodium chloride was reabsorbed during dehydration than during water loading. The medullary-cortical osmotic gradient during dehydration suggests

that a counter-current concentrating mechanism exists (Skadhauge and Schmidt-Nielsen, 1967b).

When the chicken is given a salt load intravenously, an initial diuresis occurs followed by a return to the initial value or below depending on the intensity of the salt load (Korr, 1939; Dantzler, 1966; Skadhauge and Schmidt-Nielsen, 1967a). Korr (1939) found an increase and then a decrease in GFR paralleling the change in urine flow whereas Dantzler (1966) reported only a decreasing GFR was observed and Skadhaughe and Schmidt-Nielsen (1967a) were unable to demonstrate any major changes in GFR. Dantzler (1966) observed during continuous infusion of hypertonic sodium chloride a rising urine and plasma osmolality (urine osmolality was always less than plasma osmolality) and an increase in the percent of filtered sodium excreted. Korr (1939) was able to maintain a diuresis and GFR when infusing a hypertonic solution at or above the urine flow rate, but Dantzler was not. After infusion of 15 milliequivilents sodium per kilogram, the rooster excreted a slightly hypertonic urine with a high rate of excretion of sodium for the first hour followed by an increasing rate of potassium excretion (Skadhauge and Schmidt-Nielsen, 1967a).

A number of studies have been conducted on avian species other than the chicken to examine their ability to cope with dehydration and/or salt loads (intravenous or drinking).

Primarily these investigations used birds adapted to either arid or marine environments. The budgerygahs are small (30-40 grams) birds living in the arid interior of Australia. When given 0.2 or 0.3 molar saline they tend to stop drinking and can survive without water for many days (some individuals as long as 130 days) with little loss in body weight (Cade and Dybas, 1962). The apparent physiological response to dehydration is a slight reduction in GFR (27%), an increase in filtered water reabsorbed to greater than 99%, and an increase in filtered solute excreted (Krag and Skadhauge, 1972). In water balance studies using Gambel's, California, and Bobwhite quail which inhabit desert, semidesert, and humid areas, respectively, differences in the ability to cope with dehydration and salt loads were observed (McNabb, 1969a and b). The Gambel's quail has a lower minimum requirement for water, higher tolerance for saline drinking solutions, more stable plasma osmotic pressures, greater urine to plasma osmotic pressure ratio and a larger proportion of medullary tissue in the kidney than the Bobwhite quail, with the California quail generally intermediate. Carey and Morton (1971) found that in addition to being able to process higher concentrations of seawater and saline, and produce more concentrated urine, the Gambel's quail also adapts to arid conditions behaviorally (by reducing locomotor activity) during heat stress further reducing water loss.

The savannah sparrows are able to withstand water deprivation and drink seawater to varying degrees (Cade and Bartholomew, 1959). The beldingi, a race of savannah sparrows, is restricted to salt marshes and can produce urine with higher urine to plasma ratios for osmotic pressure and chloride than can the brooksi which breeds in fresh water marshes (Poulson and Bartholomew, 1962). The beldingi can also tolerate higher plasma osmotic pressures. Poulson (1965) attributes the ability to produce a more concentrated urine to a greater proportion of medullary tissue in the kidney, i. e., more nephrons with loops of Henle. This was essentially confirmed by Johnson and Mugaas (1970a).

Many birds from marine environments, and some from the desert, possess functional salt secreting glands, nasal glands (Sturkie, 1965). The ducks and gulls have probably been the most widely studied of these birds. The nasal gland is necessary for the survival of ducks maintained on hypertonic saline drinking water, i. e., the kidney cannot handle the osmotic load by itself (Bradley and Holmes, 1972). Following acute salt loading with either hypertonic saline or seawater, there is a diphasic, renal and extrarenal, response in gulls (Douglas, 1970) and ducks (Holmes, et al., 1961). During the renal phase, there is an increase in sodium excretion followed by a decline. In the gull the sodium excretion can decrease below the initial rate.

The changes in sodium excretion are paralleled by changes in urine flow rate and sodium concentration. The extrarenal phase begins within one hour in the duck and 30 minutes in the gull. It has been shown that a major proportion of the excreted sodium load leaves via the extrarenal route in gulls (Schmidt-Nielsen, 1960; Douglas, 1970; Hughes, 1970) and ducks (Holmes et al., 1961; Holmes et al., 1968). Hughes (1970) further suggests that in the gull a major portion of the sodium and potassium was excreted by the nasal gland in the absence of an osmotic stress. It has been shown that the kidney is the primary pathway for K, Mg, NH_4, Ca and PO_4 excretion during osmotic stress (chronic and acute) whereas for Na the kidney is the minor pathway (Douglas, 1970; Holmes et al., 1968).

The role of the adrenal

Corticosterone has been measured in the adrenal venous and/or peripheral plasma of the chicken (Phillips and Chester-Jones, 1957; Nagra et al., 1960; Urist and Deutch, 1960; Resko et al., 1964; Frankel et al., 1967c; Taylor et al., 1970), the turkey (Brown, 1961; Nagra et al., 1960), the pheasant (Nagra et al., 1960), the duck (Boissin et al., 1966; Boissin, 1967; Macchi et al., 1967; Donaldson and Holmes, 1965; Bayle et al., 1971), the quail (Bayle et al., 1971), the pigeon (Bayle et al., 1971), and the gull (Macchi et al., 1967). The relative merits of the techniques used has been discussed in two recent

reviews (Frankel, 1970; Sandor, 1972). Frankel et al. (1967c) and Taylor et al. (1970) have determined the concentration of corticosterone in adrenal venous plasma to be 7.3 and 8.0 mg/100 ml, respectively, in the chicken. In the duck the secretion rate for corticosterone is 2.43 μg/minute/kg (Donaldson and Holmes, 1965).

Phillips and Chester-Jones (1957) were the first to measure aldosterone in the adrenal effluent blood of chickens. Subsequently, Taylor et al. (1970) and Sandor (1972) using the sensitive double isotope derivative assay have determined the concentrations to be 0.21 (adrenal venous) and 0.014 (peripheral) mg/100 ml plasma in the chicken and duck, respectively.

DeRoos (1960, 1961), and deRoos and deRoos (1963) were the initial investigators of in vitro corticosterone and aldosterone synthesis by the avian adrenal. Donaldson et al. (1965) established that 18-hydroxy-corticosterone is also an adrenal secretory product in vitro. Numerous other investigators have used in vitro preparations of avian adrenal to study steroidogenesis and/or steroidsecretion (see Frankel, 1970; Sandor, 1972). Steroidogenic pathways in the avian adrenal are only partially understood. Sandor (1972), based on the work of many investigators, has proposed a schema (Figure 1) for the biosythesis of adrenal steroids. One should bear in mind that this is a composite schema using data from different avian

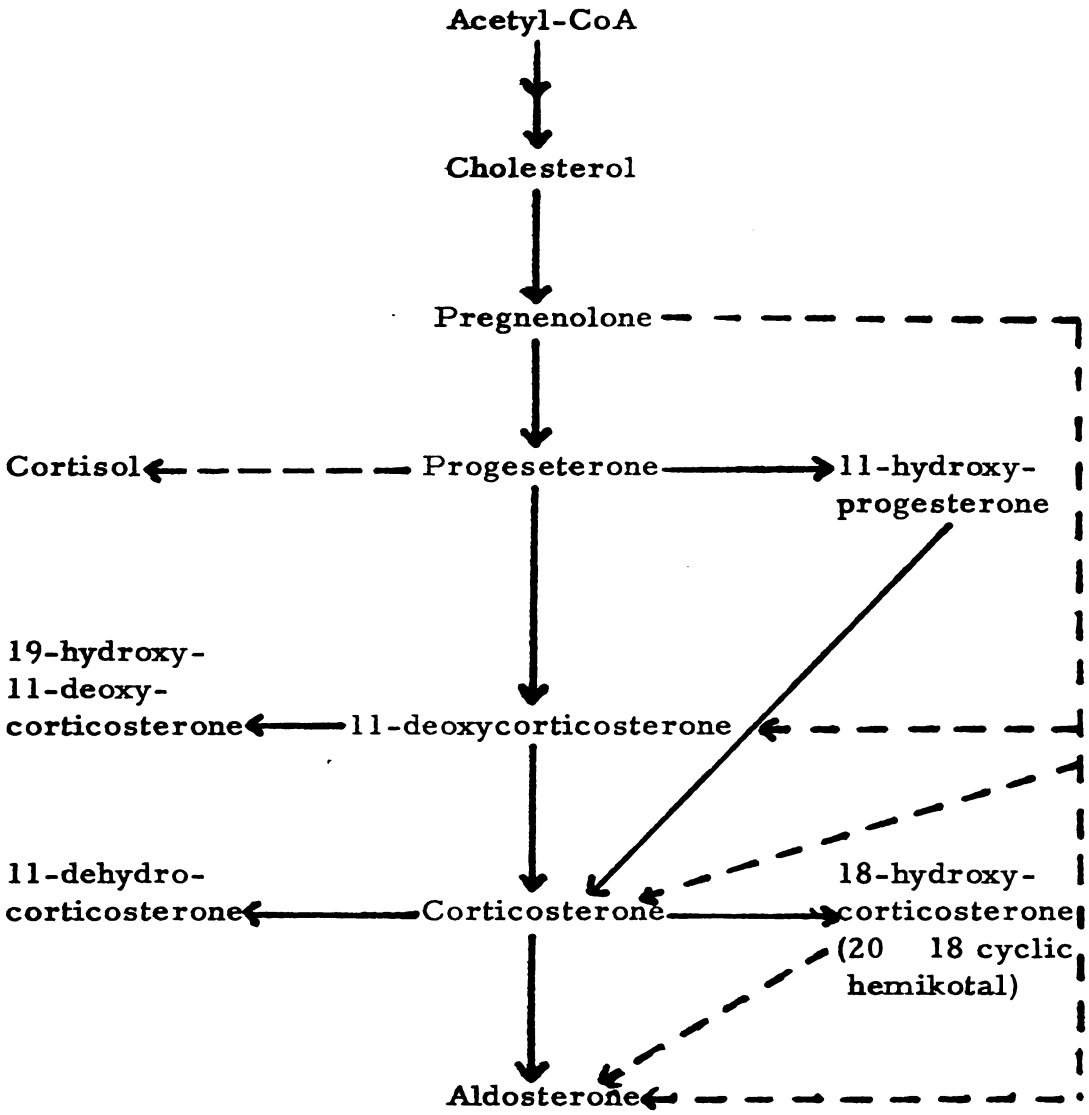

Figure 1

Biochemical pathways of steroidogenesis
in the class Aves

Note: The solid lines represent validated pathways whereas the broken lines are postulated pathways.

species and gathered in vitro. In vivo validation is still forthcoming.

Many researchers have adrenalectomized avian species including the chicken, the duck, and the pigeon (see Sturkie, 1965). Herrick and Torstyeit (1938) reported that chickens could be maintained as long as 82 days after a few injections of cortical extracts with continuous 1.6% saline drinking water and normal saline administered intramuscularly. Others have not been successful with NaCl in the drinking water (Taber et al., 1956). Deoxycorticosterone acetate (DCA), cortisone, cortisol and cortical extracts have been used to maintain avian species after adrenalectomy (Bulbring, 1940; Miller and Riddle, 1943; Brown et al., 1958b; Phillips et al., 1961). There has been little concern with electrolyte excretion following adrenalectomy. Brown et al. (1958b) made the surprising observation that following adrenalectomy sodium excretion decreased, potassium excretion showed no change in the chicken and urine volume remained normal. Serum sodium was slightly depressed whereas potassium was significantly increased. DCA (4 mg/kg/day) did not, whereas cortisone (10 mg/kg/day) did, restore sodium excretion. Serum sodium and potassium concentrations were restored by DCA and cortisone treatment. In the duck, adrenalectomy appeared to increase sodium and water excretion after saline loading (Phillips et al., 1961). When

cortisol (5mg/day) was administered, these changes were corrected.

Holmes and his collaborators have studied the effects of exogenous adrenocortical hormones on total renal excretion of sodium and potassium in the saline-loaded and water-loaded duck, a bird with a functional nasal gland. The ducks were treated with 5 mg (im) of cortisol and deoxycorticosterone at 12 and 1.5 hours and 250 μg (im) of aldosterone at 1.5 and 0 hours prior to saline loading (Holmes et al., 1961). Cortisol decreased sodium excretion but did not affect potassium excretion or urine volume during the osmotic diuresis. Deoxycorticosterone had no significant overall effects on the diuresis whereas aldosterone produced striking negative effects on urine volume, sodium output, and potassium output. In another experiment, the ducks were given 25 milliliters of distilled water at 0, 1.5, 3.0, and 4.5 hours and injected intramuscularly with corticosterone (1.25 or 2.50 mg), cortisol (1.25 or 2.50 mg), or aldosterone (50 or 100 μg) at -1.5, 0, 1.5, 3.0 and 4.5 hours (Holmes and Adams, 1963). Increases in urine volume and potassium excretion accompanied by a decline in sodium excretion were observed in birds treated with cortisol and corticosterone. Aldosterone caused highly significant retention of sodium and potassium with no effect on urine volume. In the chicken, Brown et al. (1958a) increased daily urine volume using

the unnatural steroids DCA and cortisone but observed contrasting results on sodium and potassium excretion. A decline in daily renal sodium (significant) and potassium (borderline) output was observed in birds treated with DCA (4mg/kg/day) but cortisone (15mg/kg/day) increased both significantly. Orloff and Burg (1960) briefly state that aldosterone, 2-methyl-92-fluorohydrocortisone, and desoxycorticosterone did not affect electrolyte excretion when administered into the portal system though no data, dosages, or procedures were presented. No change in the adrenal venous concentration of corticosterone or aldosterone was observed after sodium depletion of the chicken (Taylor et al., 1970). A size increase in the peripheral zone of the adrenal was reported by these authors. The biological half-lives and apparent volumes of distribution of exogenous corticosterone and aldosterone were 8.25 minutes and 780 ml/kg for corticosterone, and 8.34 minutes and 6400 ml/kg for aldosterone; these were unaffected by low or high dietary sodium intake in the duck (Thomas and Phillips, 1972).

The regulation of avian adrenal cortical function through the hypothalamus and adenohypophysis is similar to that in mammals with one notable exception (see Frankel, 1970, and Wells and Wight, 1971 for reviews). After adenohypophysectomy the concentration of corticosterone in the plasma is reduced but not to the extent which it is suppressed in mammals. Lines of

evidence have been advanced that indicate there is an extra-hypophyseal source of corticotrophin-like activity and this source is in the hypothalamus (e.g., Frankel et al., 1967b; Salem et al., 1970a, b; Bayle et al., 1971; Bouille and Bayle, 1973). Bradley and Holmes (1971) found no need to suggest the presence of this extrahypophysial source in the adenohypophysectomized duck.

In contrast to corticosterone very little is known about the regulation of aldosterone secretion by the avian adrenal. In vitro, deRoos and deRoos (1963) were able to stimulate aldosterone production with ACTH but not angiotensin II. Early work by Phillips and Chester-Jones (1957) indicated that ACTH increased aldosterone in the adrenal effluent blood (in vivo), but this was not substantiated by Taylor et al. (1970) using a more sensitive and specific assay. Following hypophysectomy the biological half-life is prolonged and the metabolic clearance rate diminished for aldosterone in the duck (Bradley and Holmes, 1971) and pigeon (Chan et al., 1972). Bayle et al. (1971) observed no change in the adrenal content of aldosterone in the hypophysectomized quail, duck, and pigeon. In the chicken, hypophysectomy and ACTH have no effect on aldosterone in the adrenal effluent plasma (Taylor et al., 1970).

Many investigators have demonstrated the presence of a renin-angiotensin system in Aves (Bean, 1942; Schaffenburg

et al., 1960; Capelli et al., 1970; Chan and Holmes, 1971; Nolly and Fasciolo, 1972, 1973). Hemorrhage increased plasma renin activity (Chan and Holmes, 1971). Following hypophysectomy, these authors observed an increase in plasma renin activity but renin plasma substrate decreased. Sodium depletion was associated with an increased granulation of the juxtaglomerular cells and increased renin content of the kidney but no change in adrenal plasma aldosterone content (Taylor et al., 1970).

The role of the neurohypophysis

Neurohypophysectomy produces polydipsia and polyuria in the chicken (Shirley and Nalbandov, 1956), and the duck (Wright et al., 1967; Bradley et al., 1971). Bradley et al. (1971) also observed increased sodium, chloride, and total osmotic activity excretion rates but no change in potassium excretion rates. Hypothalamic lesions produced similar results accompanied by a decrease in neurohypophysial pressor activity (Koike and Lepkovsky, 1967). The neurohypophysial hormones in aves are vasotocin (8-arginine oxytocin) and oxytocin (Munsick et al., 1960; Munsick, 1964), and/or vasotocin and mesotocin (Archer, 1971). In the hydrated chicken, vasotocin produced an antidiuretic effect (Munsick et al., 1960). Bradley et al. (1971) were able to reverse the effects of neurohypophysectomy (see above) with vasotocin but arginine vasopressin only returned the urine flow to normal. Skadhauge (1964) has shown

that vasotocin exerts its antidiuretic effect on the renal tubule (GFR and renal plasma flow were unaffected) from the serosal side. Blood levels of vasotocin have been reported in the chicken (Douglas and Sturkie, 1964; Sturkie and Lin, 1966) and in the chicken, quail, and pigeon (Niezgoda and Rzasa, 1971). Niezgoda and Rzasa observed higher blood levels of vasotocin in the males (all species); they suggested this might account for the higher water intake by hens observed by Lifschitz et al. (1967). An increase in vasotocin during oviposition has been reported (Sturkie and Lin, 1966); Sykes (1971) observed a delay in the diuresis due to water loading at this time. Though there are no reports on vasotocin in the circulation following the appropriate osmotic stimuli, there are reports on its depletion from the neurohypophysis following dehydration and salt loading (e. g. Lawzewitsch and Sarrat, 1970; Follet and Farner, 1966).

Other considerations

The work by Ensor and his collaborators indicate that prolactin is involved in the mineral and water metabolism of the duck (Ensor and Phillips, 1970, 1972; Ensor et al., 1973). They have established that it acts as an antidiuretic and is involved in nasal gland functions.

McNabb et al. (1973) have demonstrated that sodium and potassium are excreted in the urate precipate fraction of the chicken's urine in significant quantities. This may aid in their

excretion by reducing their contribution to osmotic pressure. The role of peritubular oncotic pressure has been investigated in the chicken using the Sperber technique (Vereerstraeten and Toussaint, 1965, 1968). They concluded that the oncotic pressure has an antinaturetic effect and therefore plays a role in sodium transport by the renal tubules.

The Cloaca, Colon, and Ceca

Investigators as early as Winer (1902) suggested that water may be reabsorbed by the colon. Korr (1939) presented data indicating that post renal isosmotic reabsorption could occur. It was observed that birds with exteriorized ureters and colostomies required 1% sodium chloride in the diet and showed a greater rate of weight loss during dehydration than intact birds but water consumption was not greater (Hart and Essex, 1942); Dixon (1958) did not observe high water intake or excretion in chickens with exteriorized ureters or colostomies. In contrast to these observations, Dicker and Halsam (1966) recorded a 64% increase in water intake after exteriorization of the ureteral openings. In another experiment, those authors observed a smaller increase (25%) which could be attributed to urine loss (Dicker and Halsam, 1972). Numerous researchers have shown that urine moves retrogradedly from the cloaca into the colon and ceca (Koike and McFarland, 1966; Akester et al., 1967; Nechay et al., 1968; Skadhauge, 1968; Ohmart et al.,

1970). Recent evidence suggests that during dehydrations post-renal reabsorption may play a significant role in water and electrolyte balance (Skadhauge, 1967, 1968; Bindslev and Skadhauge, 1971 a, b). The technique used by Skadhauge may prove to be fruitful in the investigation of the physiological importance of post-renal urine modification. Arginine vasotocin had no effect on reabsorption of water and sodium in the colon of the chicken (Skadhauge, 1967). The influence of other hormones, notably the cortical steroids, on post-renal urine formation has not been investigated.

MATERIALS AND METHODS

Experiment 1

This experiment was designed to observe the effects of corticosterone and aldosterone during the first ninety-five minutes when aldosterone and corticosterone were being infused intravenously at near estimated secretion rates. These rates were based on the work of Donaldson and Holmes (1965) in the duck on corticosterone secretion (2.43 µg/min/kg) and the ratio of corticosterone to aldosterone (40:1) in adrenal venous blood of the chicken (Taylor et al., 1970).

The experimental animals used in this study were mature Single Comb White Leghorn (SCWL) roosters. These animals were from the stocks maintained on the Poultry Science Research and Teaching Center at Michigan State University. They ranged in weight from 1.6 to 2.7 kilograms (kg). Prior to experimentation the roosters were transferred from the Center to an environmentally controlled room with 17 hours daily of light commencing at 6 A.M. and a temperature of 21°C, and were housed in individual wire cages. Feed and water were supplied ad libitum.

Prior to surgery for separating the ureters from the alimentary tract, the roosters were removed from their cages between 7:30 and 9:00 A. M. at which time they were anesthetized with 150-160 mg/kg body weight of sodium phenobarbital. No sustaining anesthesia was needed. The femoral vein was isolated through an incision in the lateral surface of the lower thigh and the m. iliotibialis. An in-dwelling cannula of polyethylene tubing (Intramedic PE 190) was inserted proximally in the incised vein approximately 4 to 5 centimeters (cm). This cannula which was filled with heparinized saline (40 units/ml) was used to collect blood samples throughout the duration of the experiment.

The rooster was restrained in the upright position on a V-shaped bird board. The tail was extended cranially and the feathers removed from the cloacal and abdominal regions. The dorsal lip of the cloaca was lifted dorsally to its limit and fastened in place with a midline stitch and two stitches were made 4-5 millimeters (mm) to either side of the midline incision. The cloaca was temporarily held open with two pair of Allis intestinal tissue forceps (6 inch) attached laterally to the lip of the cloaca. A suture was passed through the dorsal portion of the uro-proctodeal fold in the midline. This suture was used to fasten this fold dorsally, thus exposing the uretral openings. With this technique the uretral openings are partially turned

toward the midsaggital plane and the urine flows into a midline trough formed by stretching the uro-proctodeal fold dorsally. Any fecal matter in the coprodeum was then removed. The coprodeum was filled with cotton and closed by cross-stitching the coprourodeal fold. In order to continuously wash urine from the uretral openings a section of polyethylene tubing (Intramedic PE 190) was fastened in the midline dorsal to the uretral openings. Water running from this tube would flow ventrally through the trough formed from the urodeal-proctodeal fold. The urine was washed into tared centrifuge tube with deionized distilled water delivered by Harvard Infusion/Withdrawal pump (Model 940, Harvard Apparatus Co., Inc., Millis, Mass.) at a rate of 0.39828 grams per minute. Starting at the ventral midline the cloacal lip was sutured over a Teflon funnel 3 cm x 4 cm, I. D. x O. D. The sutures were placed at 5 mm. intervals alternately to the left and right until slightly over 180 degrees of the circle was fastened to the cloacal lip. Using this procedure each rooster was prepared for urine collection.

The right brachial vein was isolated in the humoral region and cannulated proximally with polyethylene tubing (Intramedic PE 60) for the infusion of ^{14}C-inulin (see below). The cannula was attached to a 3-way non-pyrogenic plastic stop-cock and filled with heparinized saline (40 units/ml) and inserted proximally into the incised vein approximately 6 cm. If blood

could be withdrawn easily, the cannula was considered to be in the vena cava; if not, it was inserted a few more millimeters until blood could be withdrawn easily. In a similar manner the left brachial vein was cannulated for infusion of the adrenal cortical hormones (see below).

Urine was collected for ten minute periods in tared polyethylene centrifuge tubes (15 x 120 mm). Immediately after collection, the centrifuge tube was sealed with Parafilm and stored at room temperature until the end of the experiment. At this time, the tubes containing the urine were weighed on a Mettler analytical balance to the nearest 0.1 milligram (T_2 in formulas below). These tubes were centrifuged at 1500 rpm for ten minutes in an International Centrifuge (Model SBV) to separate the precipitated urates from the liquid phase of the urine. The supernatant was decanted into a 1 dram screw cap vial made of borosilicate glass and capped. The centrifuge tube containing the precipitate (T_3 in formulas below) was allowed to drain dry on a paper towel for 24 hours before being weighed again. Using the following formulas it was possible to determine the volume of the urine (assuming 1 gram = 1 milliliter), and the dilution factor for the urine:

$$V\ (ml/min) = [(T_2 - T_3) - 3.9828] / 10\ min.$$

$$\text{Dilution Factor} = (T_2 - T_3)/[(T_2 - T_3) - 3.9828]$$

For subsequent determination of glomerular filtration rate

(GFR), 0.2 ml of the supernatant was set aside. The remainder was stored in a freezer at -18.5°C until sodium (Na^+) and potassium (K^+) analyses could be conducted.

Inulin clearance (C_{in} = GFR) was determined using ^{14}C-inulin (Amersham/Searle Corporation). A stock solution of ^{14}C-inulin was made to contain two microcuries (μ Ci) per ml. in 0.9% saline with 1.0% of benzyl alcohol. Each subject was given a priming dose consisting of 0.9 μCi/Kg body weight of ^{14}C-inulin from the stock solution through the cannula in the right brachial vein. Through the same cannula a ^{14}C-inulin infusion of approximately 0.007 μCi/min/Kg was maintained until the end of the experiment at 0.16 ml/min using a Harvard Compact Infusion Pump (Model 975). A minimum of 60 minutes was allowed before inulin clearances were measured. At the midpoint of the ten minute clearance period 2.5 ml of blood were removed and heparinized through the cannula in the right femoral vein and 2.0 ml of 0.9% NaCl solution were replaced through the same cannula. The blood was placed in a tapered polyethylene centrifuge tube and centrifuged at 1500 rpm for ten minutes in an International Centrifuge (Model SBV). This was transferred to a one dram screw cap vial; 0.2 ml of plasma were pipetted into a scintillation vial containing 10 ml of Bray's solution (Table 1) and the remainder was stored with the urine (see above) until Na^+ and K^+ analyses could be conducted. A Model 724 or 6848

Nuclear Chicago (Nuclear Chicago Corp., Des Plaines, Ill.) liquid scintillation counter was used to count the plasma and urine samples for ten minutes. Sample counts were corrected for background. No quenching was observed with either plasma or urine. GFR was calculated as follows:

$$GFR = C_{In} = \frac{U_{In}V}{P_{In}}$$

Where,

V = volume of urine (ml/min)

U_{In} = urine concentration of inulin ([CPM/.2 ml - Background] x dilution factor)

P_{In} = plasma concentration of inulin (CPM/.2 ml - Background)

TABLE 1

Composition of Bray's Solution

Chemical	Amount
Naphthalene	60 grams
PPO	4 grams
POPOP	200 milligrams
Methanol	100 milliliters
Ethylene glycol	20 milliliters
p-dioxane	to 1 liter

Source: Bray, 1960

The concentration of Na^+ and K^+ in the plasma and urine samples were determined with a Jarrel Ash (Model 2) Atomic Absorption Spectrophotometer using the emission mode. This unit was attached to a strip-chart recorder through a custom built signal modifying box, so that the signal could be recorded. Na^+ and K^+ determinations were made at approximately 5889 A and 7655 A, respectively, with fine adjustments, for maximum peak height, made prior to each run. The plasma and urine samples were thawed and brought to room temperature. Plasma samples were diluted 1:100 and 1:10 with deionized distilled water for Na^+ and K^+ determinations, respectively. Urine samples were diluted with deionized distilled water 1:5, 1:8, 1:10, or 1:16 (1:8 used normally). Standard solutions of Na^+ (0.2, 0.8, 1.4, and 2.0 meq/l) and K^+ (0.12, 0.48, and 1.20 meq/l) were used for determining a standard curve for each run. These standards were found to fit a simple linear regression line (Steele and Torrie, 1960) of the following form:

$$\log [\text{peak height (mm)}] = b_0 + b_1 [\text{concentration of } Na^+ \text{ or } K^+ \text{ (meq/l)}]$$

The concentration of the unknown samples was determined from the prediction equation.

Plasma Na^+, plasma K^+, urine Na^+ and urine K^+ concentration was obtained by correcting the unknown samples for dilution factor(s).

The adrenal cortical hormones used in this study were aldosterone (4-pregnen-18-al-11B, 21-diol-3, 20-dione), and corticosterone (4-pregnen-11B, 21-diol-3, 20-dione). They were obtained in pure form from Schwartz/Mann Chemical Corporation. Solutions of 5.0 mg/ml and 0.1 mg/ml in 95% ethanol were prepared from corticosterone and aldosterone, respectively. These solutions were further diluted with 95% ethanol so that when infused into the left brachial vein at a flow rate of 0.02 ml/min with a Harvard infusion/withdrawal pump (Model 950) they would be delivered at 0.04, 0.08, 0.12 and 0.16 μg/min for aldosterone, and 3.0, 6.0, 9.0 and 12.0 μg/min for corticosterone.

Ninety minutes after surgical preparation and inulin priming, each rooster was infused with either aldosterone, corticosterone, or 95% ethanol (control) for 95 minutes. During this time, urine was collected for nine ten minute clearance periods with no urine collection between 50 and 55 minutes so that urine wash and ^{14}C-inulin syringes could be refilled. Four or five roosters were used for the test substances at each level. Measured and derived parameters for the clearance periods are shown in Table 2.

TABLE 2

Parameters Measured in Experiments 1 and 2

Parameter	Symbol	Experiment
Glomerular filtration rate	$GFR = C_{In}$	1, 2
Urine flow rate (fluid)	V	1, 2
Plasma concentration of X	P_X	1
Urine (liquid phase) concentration of X	U_X	1, 2
Urine (liquid phase) excretion rate of X	E_X	1, 2
Filtered load of X	F_X	1
Clearance of X	C_X	1
Percent F_X excreted	$\%E_X$	1
Percent water reabsorbed	$Reab_{H_2O}$	1, 2
Change in body weight	ΔBW	2
Solid urine (urates)	Ur	2
Urine (solid phase) excretion rate of X	---	2
Urine (total) excretion rate of X	---	2
Percent of X excreted in solid urine	---	2

Experiment 2

This experiment was designed to test the effects of corticosterone and aldosterone on the renal function of the adult male chicken when administered intramuscularly at four times the estimated secretory rate (see Experiment 1) for three days. Since birds excrete part of their urine in solid form (urates), the comparison of the sodium and potassium excretion in the liquid portion of urine was to be made with that in the total urine (liquid and solid) to aid in the interpretation of the results from Experiment 1 (in which only liquid urine was measured).

The roosters used in this experiment were selected from the stocks maintained on the Poultry Science Research and Teaching Center. They were Single Comb White Leghorn of the Ghostley strain and were between 12 and 18 months of age. Prior to use they were brought to the cage room in Anthony Hall, weighed and maintained on a layer ration in wire floored batteries until the experiment was terminated. The light regime in the Anthony Hall location was 14.5 hours of light daily commencing at 6:30 A.M.

The protocol for this experiment called for ten birds to be divided into three experimental groups. Two groups contained three birds each; one of these was treated with .35 mg/kg/day of d-aldosterone (CIBA Pharmaceutical Corp.; Summit, N.J.) and the other with 14.0 mg/kg/day of corticosterone

(National Biochemical Corp.). The aldosterone was carried in safflower oil (.35 mg/ml) and administered intramuscularly four times daily at equal intervals alternating between the right and left thighs, starting at 8:00 A.M. of day 1 and ending at 8:00 A.M. of day 4 (72 hours later). The corticosterone was administered as a stabilized suspension (10 mg/ml) in 0.9 percent saline with 1 percent methylcellulose and following the same schedule as the aldosterone group.

The third group contained four birds and acted as the control group. One half of these birds were treated with the aldosterone carrier and the others with the corticosterone carrier on the schedule described previously. Treatments were assigned randomly to days since only one bird could be prepared for urine collection per day. The birds were weighed at 8:00 A.M. of day 1 and day 4. Feed was removed after the lights went off on the last day of treatment to aid in the surgical preparation for urine collection.

Between 10:00 and 11:00 A.M. of day 4, the birds were anesthetized with sodium phenobarbital (120-160 mg/kg) and received 3-4 μCi/kg of ^{14}C-inulin (2 μCi/ml) in 9.0 percent saline. They were prepared for urine collection as described in Experiment 1. Approximately 90 minutes following anesthetization, a 30 minute urine collection was made. The urine was washed from the funnel with deionized distilled water delivered

by a Harvard infusion pump at the rate of 10.8186 gm/30 minutes into a tared, to the nearest 0.1 mg, borosilicate glass test tube (18 x 150 mm). Care was taken to make sure no solid urine was adhered to the funnel at the end of the 30 minute period. Blood was withdrawn from the vena cava through a polyethylene cannula (Intramedic PE 50; Clay Adams Corp.), inserted via the left brachial vein. One milliliter was collected at 5, 15, and 25 minutes, in a heparinized 2.5 ml plastic syringe, after the start of urine collection. The blood was replaced with 0.9% saline.

The collected urine, after weighing, was cooled overnight at 5°C and centrifuged at 2500 rpm in an International Centrifuge (Model SBV) for 20 minutes. The liquid portion of the diluted urine was decanted into a borosilcate test tube, sealed and stored at 4°C until the sodium, potassium and inulin analyses were performed. The solid portion of the urine (urates) was dried overnight in an oven at 70°C and then stored in a one dram screw cap vial until sodium and potassium analyses were performed. The blood samples were centrifuged at 1500 rpm for 10 minutes in the same centrifuge. The plasma was stored in a one dram screw cap vial at 4°C until inulin analysis was performed.

The inulin concentration expressed as cpm per milliliter was determined liquid scintillation counting in Bray's solution as described in Experiment 1 using 0.1 ml samples of plasma and diluted urine. The urine concentration was corrected

for the dilution.

Sodium and potassium concentrations in liquid and solid urine were analyzed by flame emission spectrophotometry using a Il 453 Atomic Absorption Spectrophotometer (Instrument Laboratory Corp.). The analyses of the liquid urine were performed basically the same as in Experiment 1 except that the dilutions were approximately 100 fold greater due to the increased sensitivity of this spectrophotometer. In order to solubilize the urates, approximately 10 mg of solid urine were placed in a borosilicate glass test tube and 0.5 ml of analytical grade concentrated sulfuric acid was added. After the urates dissolved, 9.5 ml of deionized distilled water were added. This caused the uric acid to precipitate and is a modification of the procedure described by Porter (1963a) for the preparation of spectroscopically pure uric acid. These were then centrifuged at 2500 rpm for 20 minutes in the International Centrifuge (Model SBV) and diluted with deionized distilled water to fall into the range of the standards. The standards used were 8 to 20 x 10^{-3} meq/l for sodium and 4 to 14 x 10^{-3} meq/l for potassium. The final concentrations were expressed as meq/l for liquid urine and meq/mg for solid urine.

Statistical Analyses

The statistical design of the first experiment was a two-factor split-plot factorial design (Kirk, 1968). The

independent variables (parameters) were subjected to analysis of variance (ANOVA) to determine primarily if there was an interaction between level of hormone and time after infusion or an experiment-wide time effect. When an interaction existed, further ANOVA was used to determine which treatment levels varied significantly over time since a time trend was expected. Within those treatments which showed significant time effects a "t" test was used for mean separation. Separate analyses were conducted for the aldosterone and corticosterone data with the same set of birds acting as controls (zero level of hormone).

The second experiment was a one-factor completely randomized design (Kirk, 1968). The data were subjected to ANOVA and when significant effects were found Duncan's New Multiple Range test was used for mean separation.

RESULTS AND DISCUSSION

Plasma Sodium and Potassium

Plasma sodium concentrations (P_{Na}) remained stable throughout the duration of the experiments (Figures 2 and 3). There was a wide range of P_{Na} between groups in the aldosterone (.08 µg/min mean = 135.6 meq/l and .12 µg/min mean = 165.6 meq/l) and corticosterone treatments (9.0 µg/min mean = 141.4 meq/l and 12.0 µg/min mean = 166.2 meq/l). This did not appear to be related to treatments but to variations among individuals. The variation within individuals over time was small. Because of this stability, an average P_{Na} for each individual was used in calculating filtered load of sodium (F_{Na}) and percent of filtered load excreted ($\%E_{Na}$). Some reported mean values range from 132 to 180 meq/l in domestic chickens (Dantzler, 1966; Kravis and Kare, 1960; Skadhauge and Schmidt-Nielsen, 1967a; and Vereerstraeten and Toussaint, 1968).

Plasma potassium concentrations (P_K) were not as stable as P_{Na} during the experiments (Figures 4 and 5). Though there were variations between treatment groups these did not appear to be dose dependent. The P_K observed in these

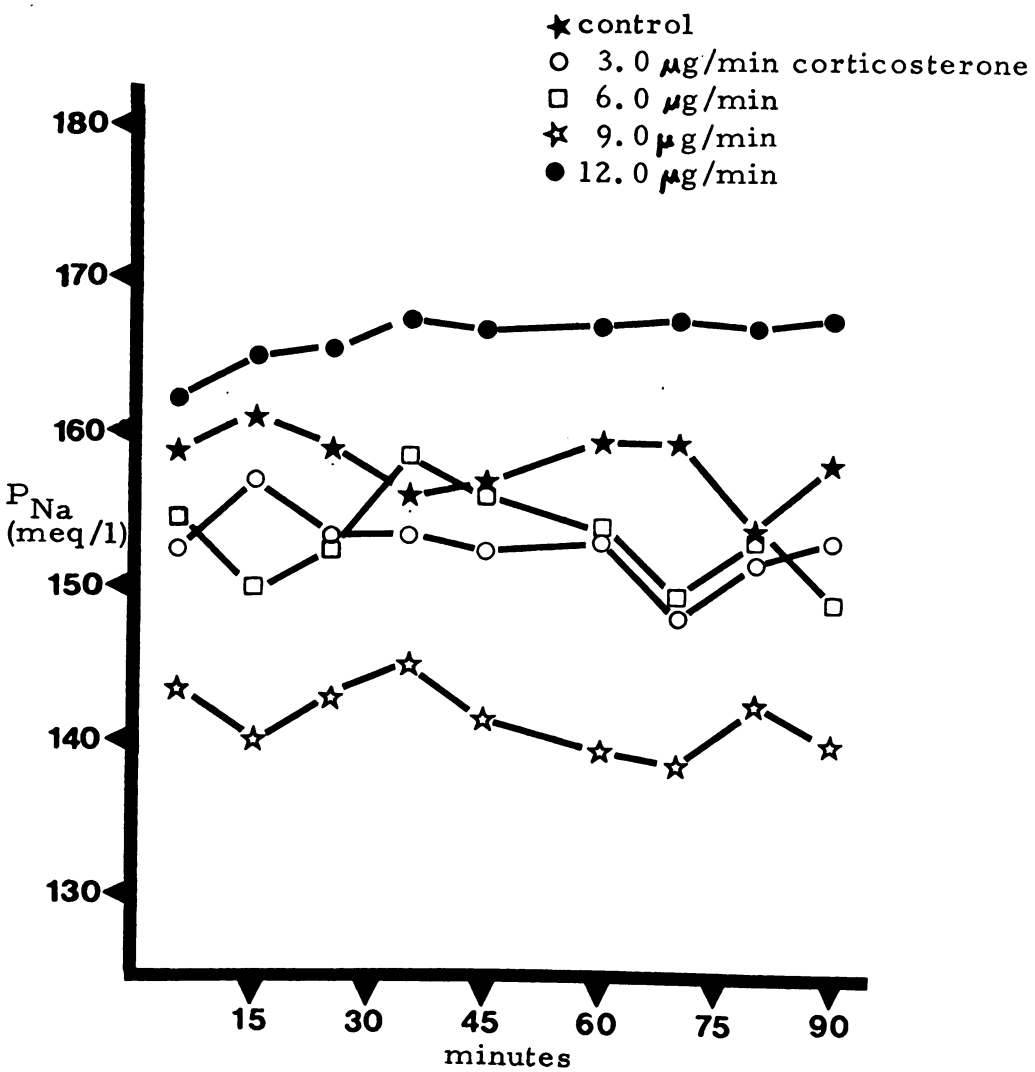

Figure 2

Plasma sodium concentrations during the 95 minutes of corticosterone infusion

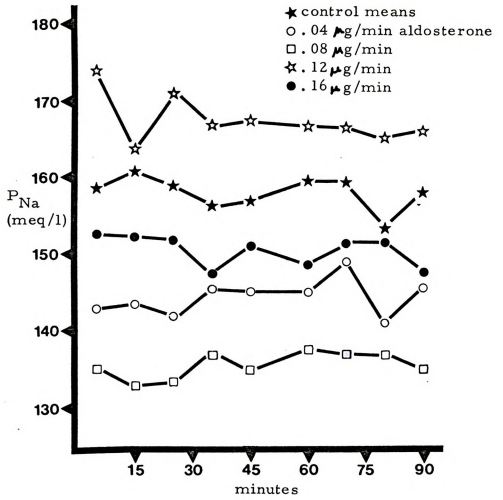

Figure 3

Plasma sodium concentrations during the 95 minutes of aldosterone infusion

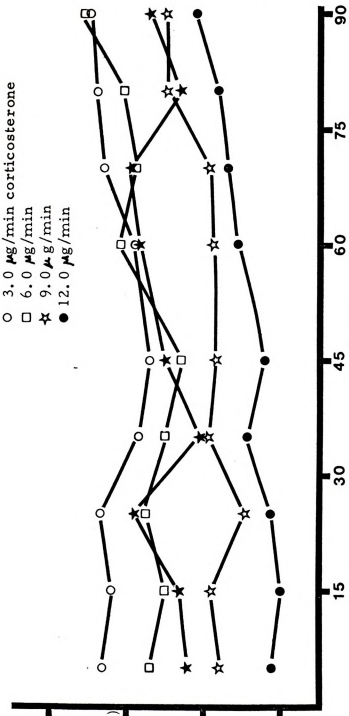

Figure 4

Plasma potassium concentrations during the 95 minutes of corticosterone infusion

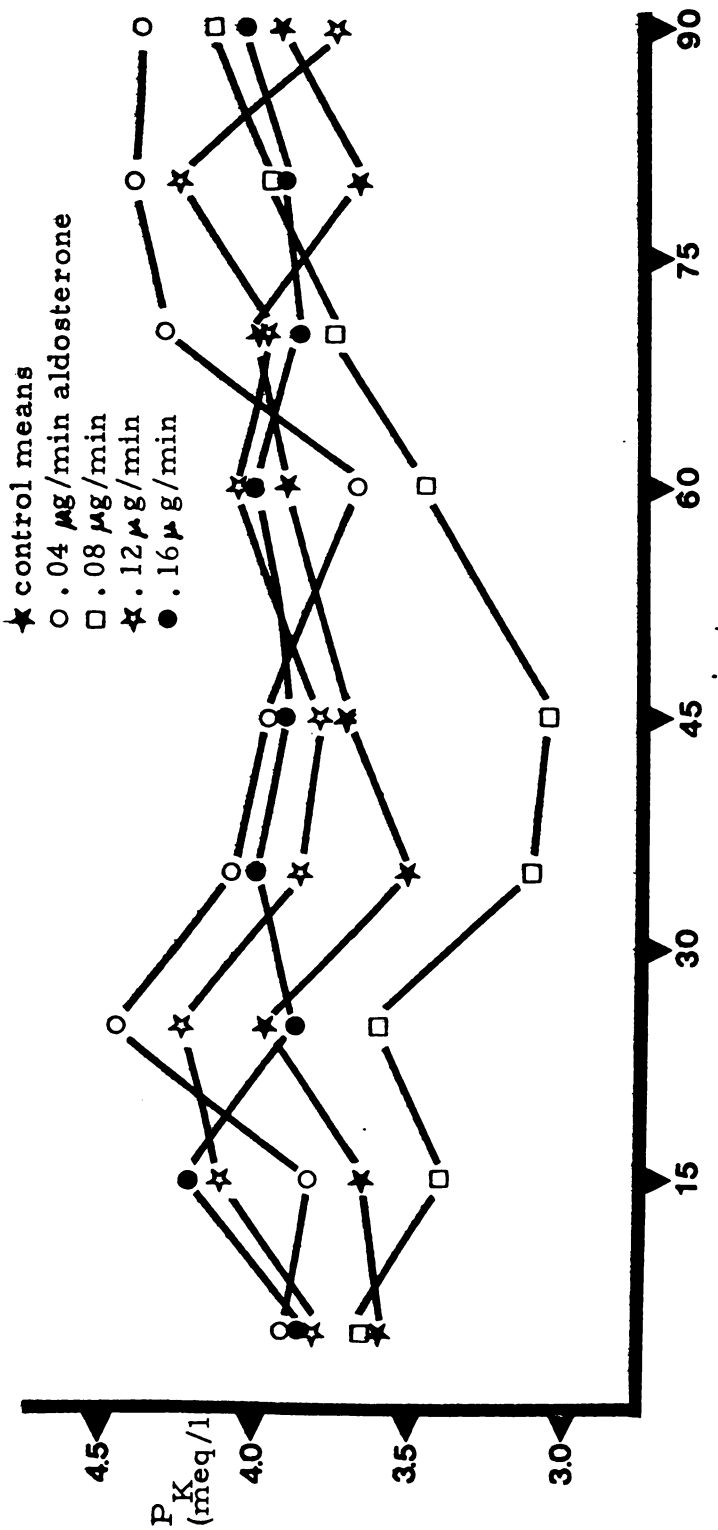

Figure 5

Plasma potassium concentrations during the 95 minutes of aldosterone infusion

experiments are compatible with those previously reported in domestic chickens (see Kravis and Kare, 1960; Orloff and Davidson, 1959; and Skadhauge and Schmidt-Nielsen, 1967a). In the aldosterone, corticosterone and control groups individuals exhibited fluctuating P_K with time. These fluctuations varied among individuals but, when all treatment groups were averaged together, P_K increased during the first 25 minutes then declined to a low at 45 minutes followed by a steady increase until the experiments were terminated (Figure 6). Analysis of variance of the means, using time as a treatment, detected no differences ($p > .10$). Due to these variations, the P_K measured at each time period was used in calculating F_K and $\%E_K$.

Glomerular Filtration Rate (GFR)

The GFR's measured in Experiments 1 and 2 are displayed in Tables 6, 7, and 8. In Experiment 1 there was considerable variation between and within individuals. Observed GFR's ranged from .62 to 4.12 ml/min/kg though most were between 1.0 and 3.0 ml/min/kg as has been reported by others (Pitts, 1938; Korr, 1939; Sperber, 1960; Dantzler, 1966; Langford and Fallis, 1966; Skadhauge, 1964; Skadhauge and Schmidt-Nielsen, 1967a). The variability within birds was often twofold and occasionally threefold. Similar findings have been reported by Langford and Fallis (1966). Both the previous authors and myself used general anesthetics whereas Skadhauge and

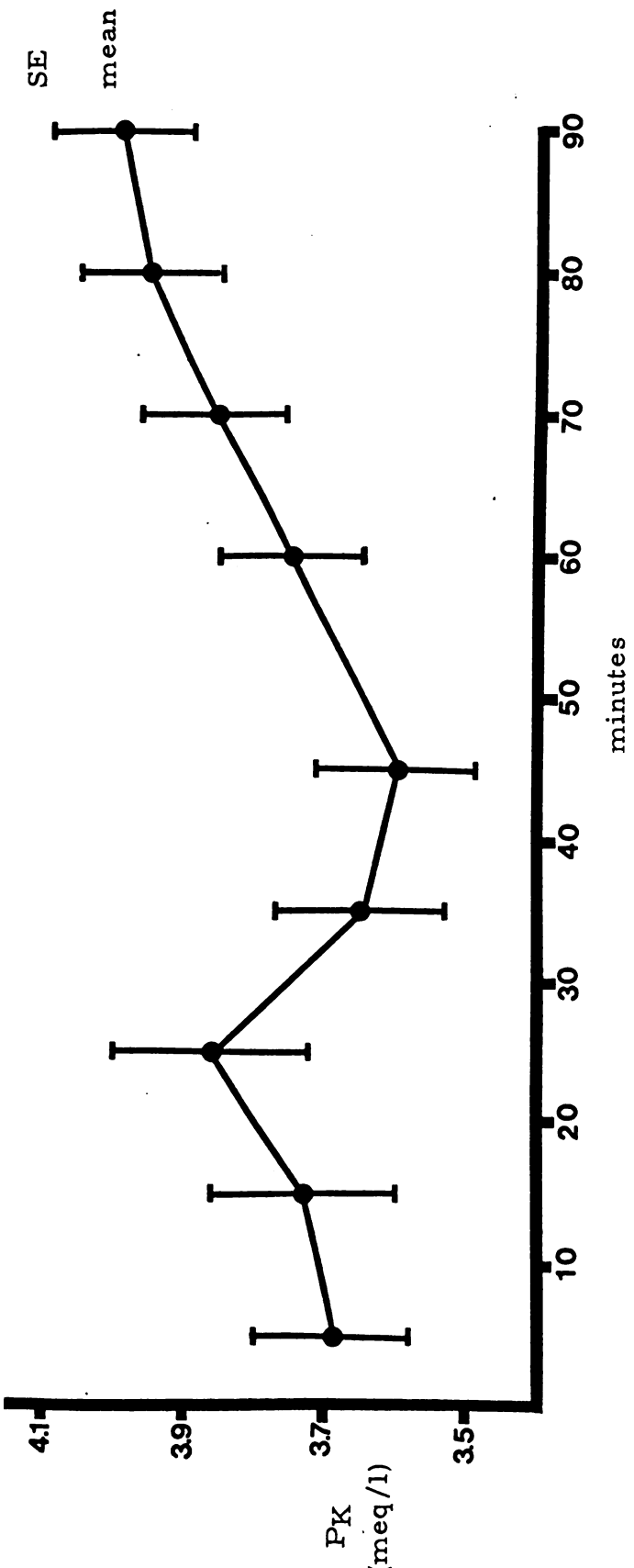

Figure 6

Mean plasma potassium concentrations of all roosters in Experiment 1

Schmidt-Nielsen (1967a), who did not report variations of this magnitude, used only local anesthetics. It is possible that the general anesthetics (barbiturates) might interfere with neural control of glomerular filtration. In Experiment 2, the range of GFRs was considerable less (1.51 to 2.60 ml/min/kg). This may have been the result of longer collection periods (30 vs. 10 minutes), less observations, and/or more homogeneity between birds.

In Table 6 can be seen the effect of corticosterone infusion on GFR. ANOVA indicated there was a significant ($p < .05$) treatment by time interaction as well as treatment and time effects (Table 3). Corticosterone at 6.0 and 9.0 μg/min caused significant increases in GFR by 55-75 minutes which then declined during the 75-95 minute period. This showed some dose dependency (Figure 7), but (paradoxically) at the highest level GFR was unaffected (Table 6).

Glucocorticoids have been shown to increase GFR in the adrenalectomized dog (Garrod et al., 1948) but not in normal men (Dingman et al., 1958). The changes seen in this experiment appeared to be transient as they were declining by the end of the experiment. This was further supported by the second experiment (Table 8) in which, after three days of corticosterone treatment, no significant change in GFR was observed.

Aldosterone had no effect on GFR in the acute

TABLE 3

Analysis of Variance Table for Selected Parameters Measured in Experiment 1 from Corticosterone Treated Adult Male Chickens

	Source	df	Mean Square				
			GFR	V	Reab H_2O	E_{Na}	$\%E_{Na}$
1.	Between subjects						
2.	A (levels of hormone)	4	8.0122**	8139.57	42.7371	2.1110	0.6586
3.	Subj. w. groups	17	1.3152	6596.16	21.1236	7.1095	0.7495
4.	Within subjects						
5.	B (periods of time)	8	.4071**	883.92**	1.7948*	0.9732**	0.0751
6.	AB	32	.1928*	424.11*	1.7943**	0.4523**	0.0976**
7.	B x subj. w. groups	136	.1091	250.07	.7537	0.2330	0.0509

CNa	E_K	$\%E_K$	C_K
107.556	3.7376	862,911	261,400.5
297.563	2.5059	521,761	155,363.6
44.410**	0.2905*	17.978	11,504.5
20.093**	0.1292	30.926	9,438.8
9.838	0.1220	25.137	7,716.8

** $p < .01$
* $p < .05$

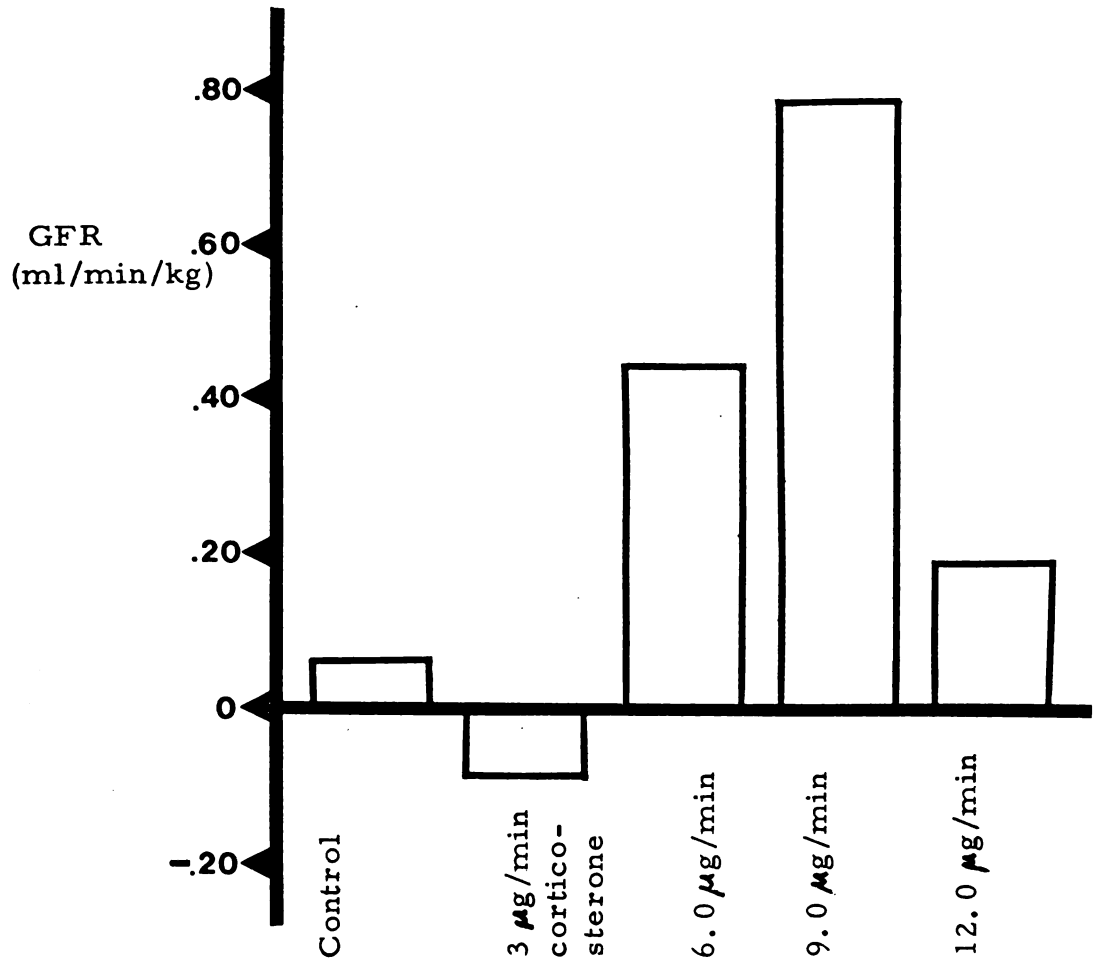

Figure 7

The effect of corticosterone infusion rates on mean changes in glomerular filtration rate at 55-75 minutes from 0-30 minutes

(Table 7) or chronic (Table 8) experiments. Others have observed that aldosterone does not affect changes in GFR when administered to dogs (Garrod et al., 1955) or man (Yunis et al., 1964; August et al., 1958). It is important to note that there was no overall effect of time in the acute experiment indicating that no significant dehydration occurred due to urinary or evaporative water loss. Korr (1939) and Skadhauge and Schmidt-Nielsen (1967a) reported reductions in GFR during dehydration; budgerygahs also showed a slight reduction in GFR during dehydration (Krag and Skadhauge, 1972).

Tubular Function

Urine flow and water reabsorption

The rate of urine formation showed a significant increase when corticosterone was infused at 9.0 and 12.0 μg/min (Tables 3 and 9). The change had a greater magnitude and occurred earlier (30-50 vs 55-75 minutes) when the higher dose was given. There was an inverse relationship between percent water reabsorbed (Reab $_{H2O}$) and urine flow (Figure 8) and the 12.0 μg/min treatment produced a significant depression in Reab $_{H_2O}$ by 30-50 minutes (Table 10). The increase in urine flow in the 9.0 μg/min group was not accompanied by any significant depression in Reab $_{H_2O}$ (Table 10 and Figure 8) but this group had the highest average GFRs and a significant increase in GFR due to corticosterone administration. This suggests that

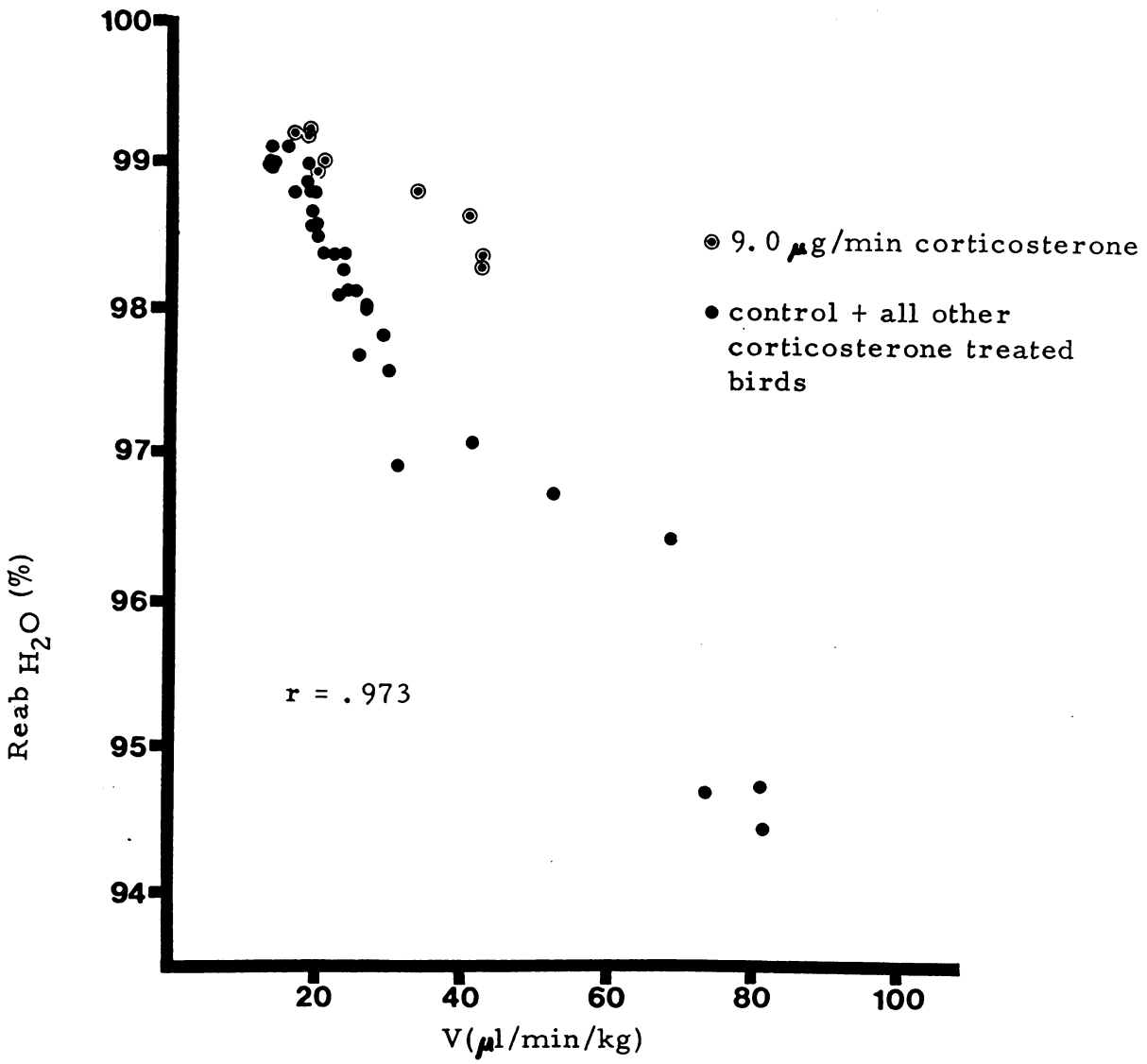

Figure 8

Relationship between urine flow rate and water resorption in control and corticosterone treated roosters in Experiment 1

the diuresis observed was due primarily to the increased filtration rate. In contrast, the diuresis observed at the higher dose appeared to be the result of a depression in water resorption. When 14 mg/kg/day corticosterone (Experiment 2) was administered i.m. for three days, a significant diuresis was produced which was due to decreased water resorption (Table 5, 8 and Figure 9).

When aldosterone was infused for 95 minutes or injected intramuscularly for three days there were no effects on urine flow or Reab $_{H_2O}$ (Tables 8, 11, 12). In the acute experiment (1) there was a significant time effect on both urine flow and Reab $_{H_2O}$ (Table 4). The rate of urine excretion began to increase at 55-75 minutes; this was accompanied by corresponding decreases in Reab $_{H_2O}$ (Tables 11 and 12). This may reflect a depression in the secretion of arginine vasotocin (ADH) due to the experimental procedure. The carrier for the steroids was ethanol (. 02 ml/min) which is known to depress ADH secretion in mammals.

Kleeman et al. (1958) and Raisz et al. (1957) suggested that high doses of glucocorticoids decreased the permeability of the distal nephron to water in the absence of ADH. In the experiments presented here the corticosterone (a glucocorticoid) could have been acting at the distal nephron since the experimental procedure may have depressed ADH secretion.

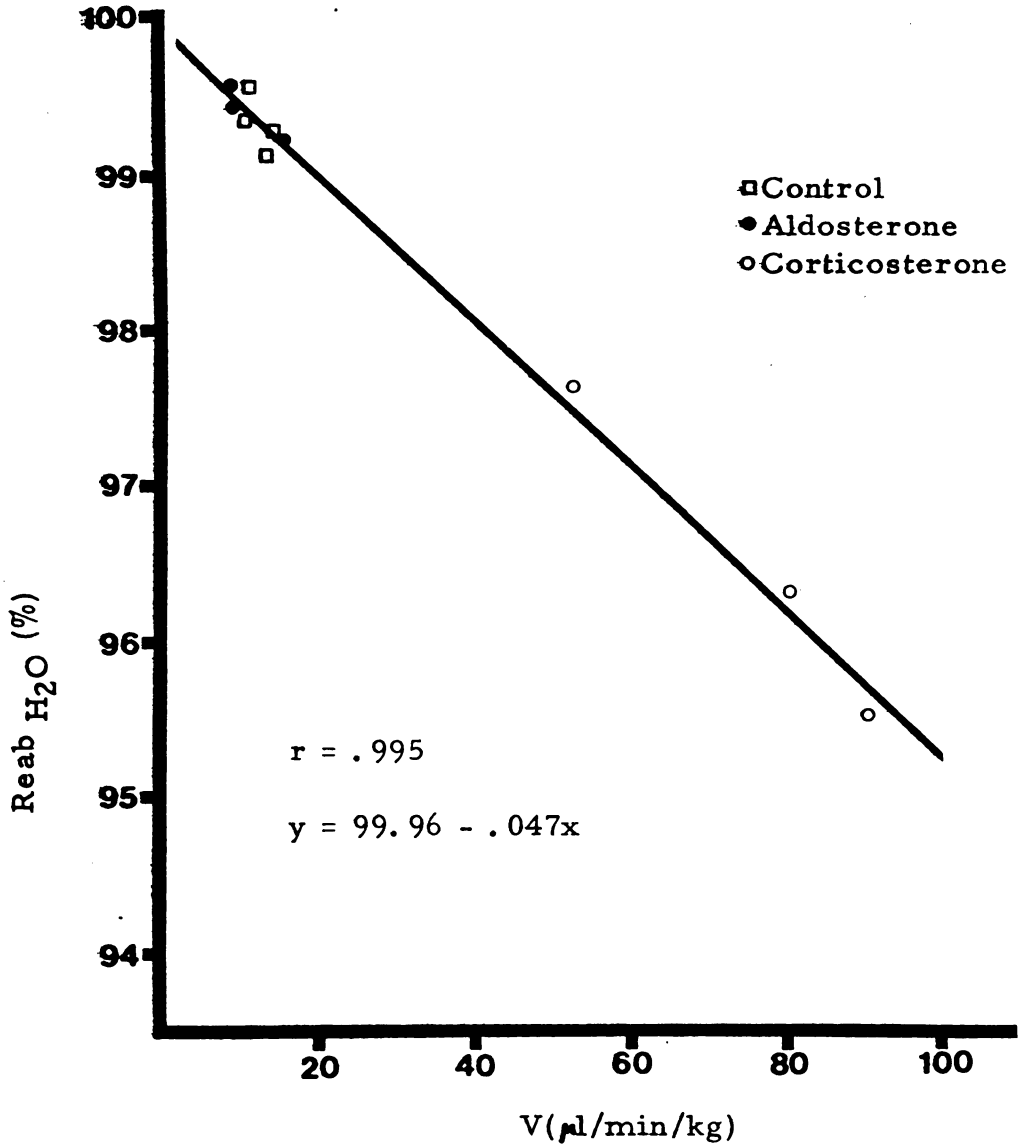

Figure 9

Relationship between urine flow rate and water resorption in control, aldosterone and corticosterone treated roosters in Experiment 2

TABLE 4

Analysis of Variance Table for Selected Parameters Measured in Experiment 1 from Aldosterone Treated Male Chickens

	df	Mean Squares				
		GFR	V	$Reab_{H_2O}$	E_{Na}	$\%E_{Na}$
1. Between subjects						
2. A (levels of hormone)	4	0.2918	1609.83	6.7685	27.4621	8.0389
3. Subj. w. groups	19	0.6238	2421.17	13.4253	20.2937	4.9392
4. Within subjects						
5. B (periods of time)	8	0.9315	124.10*	1.1143*	0.4921	0.1768
6. AB	32	0.0786	36.46	0.3465	0.6241	0.1812
7. BxSubj. w. groups	152	0.1163	38.84	0.5408	0.7873	0.2124

C_{Na}	E_K	$\%E_K$	C_K
1578.556	4.1283*	1460.363**	284,248.8*
829.946	0.9253	192.149	59,535.0
27.173	0.1823	50.255	11,979.0
34.785	0.2289**	56.666	17,414.4**
36.256	0.112	42.799	9,216.9

**$p < 0.01$
*$p < 0.05$

TABLE 5

Analysis of Variance Table for Selected Parameters Measured in Experiment 2

Source	df	Mean Squares				
		B.W.	GFR	V	Reab H_2O	E_{Na}
Treatments	2	0.0218**	0.0399	4,323.3**	9.2552**	1962.7**
Error	7	0.0023	0.1232	137.3	0.4464	178.8
		E_K	U_{Na}	U_K	Na in Ur	K in Ur
		3,993.80**	27,332**	1283.8	146.2**	30.01
		87.40	1,147	845.3	15.8	97.01
		Total Na	Total K	$\%E_{Na}$ in Ur	$\%E_K$ in Ur	
		3125.6**	4456.1**	115.02**	684.46**	
		281.4	391.8	12.31	76.96	

** $p < .01$

TABLE 6

The Effects of Corticosterone Infusion Rate on Glomerular Filtration Rate for 95 Minutes
GFR (ml/min/kg) Corticosterone*

	N	Minutes			
		0-30	30-50	55-75	75-95
Control	5	1.40±.11	1.43±.10	1.46±.07	1.61±.10
3.0 µg/min	4	1.55±.09	1.58±.20	1.46±.08	1.36±.12
6.0 µg/min	5	1.44±.11	1.62±.24	1.88±.20	1.60±.17
9.0 µg/min	4	2.27±.16^{a}**	2.12±.20^{a}	3.05±.23^{b}	2.67±.16^{ab}
12.0 µg/min	4	1.21±.12	1.34±.12	1.40±.19	1.44±.20

*Data presented as mean±standard error.

**Means within treatments with differing superscripts significantly different ($p < .05$).

TABLE 7

The Effects of Aldosterone Infusion Rate on Glomerular Filtration Rate for 95 Minutes
GFR (ml/min/kg) Aldosterone*

		Minutes			
	N	0-30	30-50	55-75	75-95
Control	5	1.40±.11	1.43±.10	1.46±.07	1.61±.10
0.04 µg/min	4	1.39±.13	1.68±.23	1.65±.12	1.53±.09
0.08 µg/min	5	1.44±.09	1.58±.20	1.49±.16	1.38±.13
0.12 µg/min	5	1.41±.07	1.34±.07	1.47±.25	1.27±.08
0.16 µg/min	5	1.36±.07	1.34±.09	1.25±.06	1.36±.06

*See footnote, Table 6.

TABLE 8

The Effects of Aldosterone (.35 mg/kg/day) and Corticosterone (14.0 mg/kg/day) Administered for Three Days on Glomerular Filtration Rate, Urine Flow Rate, Water Resorption, Urate Excretion Rate, and Body Weight Changes*

	Control	Corticosterone	Aldosterone
	n = 4	n = 3	n = 3
ΔB.W. (kg)	.025±.044^{a**}	-.073±.045^{b}	.097±.057^{a}
GFR (ml/min/kg)	1.95 ± .49	2.16 ± .12	2.04 ± .23
Reab H_2O(%)	99.33 ± .18^{x}	96.41 ± 1.22^{y}	99.43 ± .17^{x}
V (μl/min/kg)	12.4 ± 1.7^{x}	76.2 ± 21.5^{y}	11.6 ± 3.7^{x}
Solid urine (mg/30 min)	59.1 ± 1.9	77.4 ± 58.6	45.0 ± 7.8

*Data presented as mean ± standard deviation.

**Means within parameter with differing superscripts significantly different a and b ($p < .05$), and x and y ($p < .01$).

TABLE 9

The Effects of Corticosterone Infusion Rate on Urine Flow Rate for 95 Minutes
V (µl/min/kg) Corticosterone*

	N	Minutes 0-30	30-50	55-75	75-95
Control	5	25.6±4.8	21.1±5.2	24.6±4.8	23.8±4.8
3.0 µg/min	4	20.0±4.4	19.9±3.4	22.7±2.9	24.6±1.9
6.0 µg/min	5	14.0±1.3	13.6±1.6	16.8±1.8	17.5±1.7
9.0 µg/min	4	18.4±2.0[a]**	20.8±3.1[ab]	41.9±12.1[b]	38.3±11.2[ab]
12.0 µg/min	4	29.1±8.7[a]	57.6±18.9[b]	81.4±28.7[c]	57.4±26.8[b]

*See footnote, Table 6.

**See footnote, Table 6.

TABLE 10

The Effects of Corticosterone Infusion Rate on Water Resorption for 95 Minutes
Reab H_2O (%) Corticosterone*

		Minutes			
	N	0-30	30-50	55-75	75-95
Control	5	98.00±.41	98.46±.39	98.24±.36	98.35±.40
3.0 µg/min	4	98.56±.37	98.64±.25	98.38±.29	98.05±.25
6.0 µg/min	5	99.02±.08	99.11±.09	99.08±.10	98.84±.14
9.0 µg/min	4	99.19±.07	99.00±.13	98.53±.46	98.57±.39
12.0 µg/min	4	97.63±.68[a**]	95.90±1.21[b]	94.61±1.47[c]	96.61±1.23[ab]

*See footnote, Table 6.

**See footnote, Table 6.

TABLE 11

The Effects of Aldosterone Infusion Rate on Urine Flow Rate for 95 Minutes V (µl/min/kg) Aldosterone*

		Minutes			
	N	0-30	30-50	55-75	75-95
Control	5	25.6±4.8	21.1±5.2	24.6±4.8	23.8±4.8
0.04µg/min	4	28.3±7.4	33.8±10.6	33.3±9.3	39.3±10.3
0.08µg/min	5	27.5±3.6	28.5±4.9	33.4±7.0	32.0±5.0
0.12µg/min	5	19.4±2.1	17.4±1.9	19.0±1.9	23.0±2.7
0.16µg/min	5	16.8±1.3	16.8±1.1	19.2±3.4	24.1±4.1
Mean		23.5[a**]	23.5[a]	25.9[ab]	28.4[b]

*See footnote, Table 6.

**See footnote, Table 6.

TABLE 12

The Effects of Aldosterone Infusion Rate
on Water Resorption for 95 Minutes
Reab H_2O (%) Aldosterone*

	N	Minutes 0-30	30-50	55-75	75-95
Control	5	98.00±.41	98.46±.39	98.24±.36	98.36±.40
0.04 µg/min	4	98.18±.32	98.15±.42	98.09±.42	97.62±.48
0.08 µg/min	5	97.86±.35	97.90±.48	97.21±.89	97.17±.75
0.12 µg/min	5	98.65±.10	98.69±.13	98.23±.49	98.16±.26
0.16 µg/min	5	98.76±.08	98.69±.14	98.42±.34	98.24±.31
Mean		98.29^{ab}**	98.38^{a}	98.04^{ab}	97.90^{b}

*See footnote, Table 6.

**See footnote, Table 6.

Another interpretation of these data is that the corticosterone affected a change in the distribution of renal blood flow away from the nephrons with loops of Henle to those with none. Alternative hypotheses include the direct inhibition of ADH release, decreasing the renal medullary osmotic gradient, and increasing electrolyte excretion. However, there is evidence in man that glucocorticoids do not effect ADH release (Kleeman et al., 1964). In adrenalectomized rats the glucocorticoids are necessary to re-establish the lowered medullary osmotic gradient (Crabbe and Nichols, 1959) but adrenalectomy in the chicken has no effect on urine flow (Brown et al., 1958b). It will be shown by subsequent data that corticosterone had some contradictory effects on sodium excretion.

Sodium and potassium excretion

There was a significant interaction (level of hormone x time) effect on the measures of sodium excretion (E_{Na}, $\%E_{Na}$ and C_{Na}) when corticosterone was infused (Table 3). In the second experiment corticosterone had significant effects on sodium excretion (Table 5). These data are presented in Tables 13, 14, 15, and 19.

The rate of sodium excretion (E_{Na}) showed significant increase by 55-75 minutes after corticosterone infusion at 3, 6 and 9 μg/min was initiated (Table 13). This response may have been transient since E_{Na} declined during the 75-95 minute

TABLE 13

The Effects of Corticosterone Infusion Rate on the Excretion Rate of Sodium for 95 Minutes
E_{Na} (μeq/min/kg) Corticosterone*

		Minutes			
	N	0-30	30-50	55-75	75-95
Control	5	1.60±.21	1.33±.26	1.44±.12	1.97±.24
3.0 μg/min	4	0.77±.08[a**]	1.02±.25[ab]	1.76±.37[c]	1.67±.43[bc]
6.0 μg/min	5	1.55±.18[a]	1.62±.25[a]	2.30±.30[ab]	2.07±.26[ab]
9.0 μg/min	4	1.37±.24[a]	1.58±.39[ab]	2.24±.42[b]	1.30±.24[a]
12.0 μg/min	4	1.92±.43	1.77±.34	1.75±.47	1.63±.48

*See footnote, Table 6.

**See footnote, Table 6.

TABLE 14

The Effects of Corticosterone Infusion Rate on the Clearance of Sodium for 95 Minutes C_{Na} (µl/min/kg) Corticosterone*

	N	Minutes 0-30	30-50	55-75	75-95
Control	5	100±12	83±15	91± 7	125±16
3.0 µg/min	4	50±5^{a}**	66±15^{ab}	113±22^{c}	107±26^{bc}
6.0 µg/min	5	104±12^{a}	109±18^{a}	154±21^{b}	139±19^{ab}
9.0 µg/min	4	99±18^{a}	115±30^{a}	163±32^{b}	94±18^{a}
12.0 µg/min	4	115±25	105±20	105±28	97±28

*See footnote, Table 6.

**See footnote, Table 6.

TABLE 15

The Effects of Corticosterone Infusion Rate
on the Percent of Filtered Sodium
Excreted for 95 Minutes
$\%E_{Na}$ Corticosterone*

	N	Minutes			
		0-30	30-50	55-75	75-95
Control	5	0.71±.06	0.58±.09	0.64±.06	0.83±.14
3.0 µg/min	4	0.33±.04[a**]	0.45±.11[ab]	0.81±.17[b]	0.82±.19[b]
6.0 µg/min	5	0.73±.08	0.72±.10	0.83±.09	0.86±.07
9.0 µg/min	4	0.43±.07	0.55±.13	0.58±.12	0.37±.08
12.0 µg/min	4	0.92±.18	0.78±.11	0.72±.12	0.62±.11

*See footnote, Table 6.

**See footnote, Table 6.

period. This was particularly evident at the 6 and 9 μg/min treatments. C_{Na} (Table 15) followed the same trends as E_{Na} since P_{Na} was not affected by the steroids. When the excretion of sodium is weighted relative to filtration rate ($\%E_{Na}$) only the 3 μg/min corticosterone treatment exhibited a significant increase (Table 14). The observation that in treatments of 6 and 9 μg/min of corticosterone E_{Na} increased as does GFR suggest that in the rooster the excretion of sodium may be directly effected by the GFR. One of the roles of glucocorticoids in mammalian renal function is the maintainence of G-T balance (Lockett, 1972). In the mammalian kidney changes in filtered load of sodium (and GFR) are balanced by changes in proximal tubular resorption of sodium and water (Klahr and Slatopolsky, 1973). The increase in all measures of sodium excretion (E_{Na}, $\%E_{Na}$, and C_{Na}) when corticosterone is infused at 3 μg/min is indicative of inhibited sodium reabsorption at low dose levels.

In contrast to the results of the acute experiment (1), a higher dose of corticosterone given over three days caused a decrease in sodium excretion in liquid, solid (urates) and total urine (Table 19). In large quantities corticosterone and cortisol administered to ducks (Holmes and Adams, 1963) and DCA, but not cortisone acetate, administered to cockerels (Brown et al., 1958a) decreased sodium excretion. Cortisone acetate had a naturetic effect. It is important to note that, given these

experimental conditions, the liquid urine E_{Na} accurately reflected the total urine E_{Na}, since the first experiment measured the former only. McNabb et al. (1973) speculated that cation-urate interactions could play a significant role in cation excretion, particularly when challened with a large osmotic load. This probably partially accounts for the observation by Skadhauge and Schmidt-Nielsen (1967a) that the dehydrated rooster reabsorbs more filtered water at a given urine: plasma osmotic ratio than does the mammal.

In the first experiment potassium excretion was not affected by corticosterone but did show an experimentwide tendency to increase ($p < .05$) during the experimental period (Tables 3 and 20). This tendency disappeared when E_K was corrected for P_K (Table 21) and GFR (Table 22) indicating that the E_K trend was probably the result of increasing P_K during the experiment. However, after three days of corticosterone treatment (Experiment 2) E_K (total urinary and liquid urine) was significantly increased.

This is in agreement with the work of Holmes and Adams (1963) in the water-loaded duck treated with large doses of glucocorticoids (cortisone and corticosterone). However, Brown et al. (1958a) reported contrasting effects on potassium excretion in cockerels treated with DCA (decrease) and cortisone acetate (increase).

None of the measures of sodium excretion show any significant effects due to hormone treatment, time or their interaction in Experiment 1 (Tables 4, 16, 17 and 18). The most probable explanation being that the latency period was longer than 95 minutes in the chicken. These have been variously reported between 30-120 minutes (see Lockett, 1972; Knochel and White, 1973). When aldosterone was given in larger doses (Experiment 2) a significant decrease in sodium excretion was observed (Tables 5 and 19). The rate of sodium excretion reported here is in close agreement with that published for the duck (Holmes and Adams, 1963) and the male chicken (Brown et al., 1958a, 1958b; Skadhauge and Schmidt-Nielsen, 1967a). In the duck, Holmes and co-workers (1961, 1963) observed significant sodium retention when aldosterone was administered. An antinaturetic effect was observed in cockerels with DCA treatment (Brown et al.,1958a). Brown et al. (1958b) noted a decrease in sodium excretion in adrenalectomized chickens which could be corrected by cortisone acetate but not DCA.

The effects of aldosterone on potassium excretion during the first 95 minutes after intravenous infusion begins are difficult to interpret. The ANOVA indicates there is a significant level of hormone over time effect (interaction) on E_K and C_K (Table 4). The 0.08 and 0.12 μg/min treatments showed significant, and the control treatment showed nearly significant

TABLE 16

The Effects of Aldosterone Infusion Rate on the Excretion Rate of Sodium for 95 Minutes E_{Na} (μeq/min/kg) Aldosterone*

	N	Minutes			
		0-30	30-50	55-75	75-95
Control	5	1.60±.21	1.33±.26	1.44±.12	1.97±.24
0.04 μg/min	4	2.18±.63	2.05±.75	2.94±.94	2.78±.81
0.08 μg/min	5	2.91±.78	2.88±.77	2.76±.69	2.79±.88
0.12 μg/min	5	0.80±.16	0.87±.19	0.87±.16	0.90±.16
0.16 μg/min	5	1.54±.19	1.63±.15	1.70±.25	1.30±.19

*See footnote, Table 6.

TABLE 17

The Effects of Aldosterone Infusion Rate on the Clearance of Sodium for 95 Minutes
C_{Na} (µl/min/kg) Aldosterone*

	N	Minutes			
		0-30	30-50	55-75	75-95
Control	5	100±12	83±15	91 ± 7	125±16
0.04 µg/min	4	161±41	158±52	234±75	229±74
0.08 µg/min	5	191±45	189±44	185±38	185±52
0.12 µg/min	5	48 ± 9	52±11	52±10	54±10
0.16 µg/min	5	103±13	109±10	113±17	88±13

*See footnote, Table 6.

TABLE 18

The Effects of Aldosterone Infusion Rate on the Percent of Filtered Sodium Excreted for 95 Minutes
$\%E_{Na}$ Aldosterone*

	N	Minutes			
		0-30	30-50	55-75	75-95
Control	5	0.71±.06	0.58±.09	0.64±.06	0.83±.14
0.04 µg/min	4	1.04±.21	0.88±.28	1.33±.44	1.42±.47
0.08 µg/min	5	1.57±.41	1.44±.39	1.46±.42	1.50±.42
0.12 µg/min	5	0.33±.06	0.37±.07	0.52±.23	0.43±.08
0.16 µg/min	5	0.77±.10	0.85±.09	0.95±.16	0.64±.08

*See footnote, Table 6.

TABLE 19

The Effects of Aldosterone (0.35 mg/kg/day) and Corticosterone (14.0 mg/kg/day) Administered for 3 Days on the Excretion Rates of Sodium and Potassium in Liquid, Solid and Total Urine, and Liquid Urine Concentration of Sodium and Potassium*

	Control	Corticosterone	Aldosterone
	n = 4	n = 3	n = 3
U_{Na} (meq/l)	187.8±50.1^{a**}	15.9±3.2^{b}	70.2±15.7^{b}
U_{K} (meq/l)	68.4±32.9	41.1±16.2	81.5±32.7
Liquid Urine			
E_{Na} (μeq/30 min/kg)	68.3±18.8^{a}	35.2±4.3^{ab}	24.3±8.9^{b}
E_{K} (μeq/30 min/kg)	25.0±11.2^{a}	87.4±8.3^{b}	26.7±7.0^{a}
Solid Urine			
E_{Na} (μeq/30 min/kg)	13.0±6.0^{x}	1.3±0.7^{y}	2.8±0.1^{y}
E_{K} (μeq/30 min/kg)	15.6±7.7	13.2±14.5	9.7±6.3
Total Urine			
E_{Na} (μeq/30 min/kg)	82.3±24.4^{a}	36.5±3.6^{b}	27.1±9.0^{b}
E_{K} (μeq/30 min/kg)	40.6±17.5^{a}	103.9±27.1^{b}	36.5±13.3^{a}

*Data presented as mean ± standard deviation.

**Means within parameter with differing superscripts are significantly different, a and b ($p < .01$), and x and y ($p < .05$).

TABLE 20

The Effects of Corticosterone Infusion Rate on the Excretion Rate of Potassium for 95 Minutes
E_K (μeq/min/kg) Corticosterone*

		Minutes			
	N	0-30	30-50	55-75	75-95
Control	5	0.34±.03	0.38±.04	0.53±.07	0.82±.13
3.0 μg/min	4	1.07±.18	0.97±.20	0.92±.20	0.77±.15
6.0 μg/min	5	0.98±.19	0.89±.09	1.26±.20	1.42±.11
9.0 μg/min	4	1.06±.27	1.31±.33	1.53±.36	1.28±.42
12.0 μg/min	4	0.68±.07	0.78±.09	0.81±.06	0.88±.13
Mean		0.81^{a**}	0.84^{ab}	1.00^{ab}	1.04^{b}

*See footnote, Table 6.

**See footnote, Table 6.

TABLE 21

The Effects of Corticosterone Infusion Rate on the Clearance of Potassium for 95 Minutes
C_K (µl/min/kg) Corticosterone*

	N	Minutes			
		0-30	30-50	55-75	75-95
Control	5	92±6	103±9	133±14	215±33
3.0 µg/min	4	261±48	250±51	223±43	178±34
6.0 µg/min	5	258±42	244±39	311±45	343±26
9.0 µg/min	4	297±72	366±87	433±95	320±92
12.0 µg/min	4	222±20	244±22	247±25	266±51

*See footnote, Table 6.

TABLE 22

The Effects of Corticosterone Infusion Rate on the Percent of Filtered Potassium Excreted for 95 Minutes
$\%E_K$ Corticosterone*

	N	Minutes			
		0-30	30-50	55-75	75-95
Control	5	6.9±0.6	7.6±0.9	9.5±1.2	14.0±2.2
3.0 µg/min	4	17.3±3.0	17.6±3.7	14.8±2.3	12.8±2.2
6.0 µg/min	5	18.9±2.8	17.7±2.8	18.2±2.8	24.1±2.9
9.0 µg/min	4	12.2±2.5	17.4±3.8	14.3±3.2	11.3±2.9
12.0 µg/min	4	20.6±3.0	20.0±3.4	20.0±2.9	19.6±2.9

*See footnote, Table 6.

($p < .10$), time related effects for E_K. Inspection of the data in Tables 23 and 20 indicates that in the control birds E_K and C_K began increasing during the 55-75 minute period and continued until the end of the experiment whereas the 0.08 μg/min treatment caused an increase at the 55-75 minute period and began declining during the 75-95 minute period, and the 0.12 μg/min began decreasing during the 30-50 minute period becoming significant at the 55-75 minute period, and starting to rise during the last period. When expressed as a percent of filtered load ($\%E_K$) the interaction effect becomes nonsignificant (Tables 4 and 15). In Experiment 2 where aldosterone was administered for three days there was no significant effect on potassium excretion (Tables 5 and 19). These two experiments taken together led to the conclusion that aldosterone had no permanent effect on potassium excretion in the male chicken. Aldosterone treatment in ducks (Holmes and Adams, 1963) and DCA treatments in cockerels (Brown et al., 1958a) had the astonishing effect of decreasing potassium excretion. But, Brown et al. (1958b) observed no effect on potassium excretion in the adrenalectomized male chicken.

The role of aldosterone in the renal excretion of sodium and potassium has been the subject of intensive research efforts during the past twenty years, particularly in mammalian species. Typically, aldosterone promotes sodium reabsorption

TABLE 23

The Effects of Aldosterone Infusion Rate on the Excretion Rate of Potassium for 95 Minutes

E_K (μeq/min/kg) Aldosterone*

		Minutes			
	N	0-30	30-50	55-75	75-95
Control	5	0.34±.03	0.38±.04	0.53±.07	0.82±.13
0.04 μg/min	4	1.04±.20	0.89±.18	1.38±.23	1.19±.18
0.08 μg/min	5	0.77±.07[a]**	0.76±.06[a]	1.19±.23[b]	1.10±.20[ab]
0.12 μg/min	5	1.45±.15[a]	1.24±.08[ab]	0.97±.12[b]	1.07±.17[ab]
0.16 μg/min	5	0.64±.08	0.61±.09	0.71±.13	0.53±.06

*See footnote, Table 6.

**See footnote, Table 6.

and potassium excretion (Lockett, 1972; Knochel and White, 1973). Aldosterone exerts its effect primarily on the distal tubule of the nephron (Knochel and White, 1973; Schultze, 1973). These authors concluded that there is a dissociation of effects of aldosterone on sodium and potassium transport and that it is not an exchange of sodium for potassium or hydrogen as was formerly thought. In line with this hypothesis, Malnic et al. (1966), based on their micropuncture studies, proposed that there was an active resorption of sodium and potassium, and passive secretion of potassium in the distal tubule. They state that the direction and rate of potassium movement was determined by the balance of these forces. It has also been postulated that a difference in function may exist between the early and late distal tubule (Burg and Stoner, 1974). The data support the concept of separation of sodium and potassium effects. In the chronic experiment (Experiment 2) sodium retention was increased and potassium excretion unaffected. When aldosterone was infused at levels near estimated physiological secretory rates (Experiment 1) potassium excretion was sometimes affected (in one case positively and in the other negatively) without change in sodium retention up to 95 minutes. Permanent effects on the sodium retention may have been missed because the latency period for aldosterone action may have been longer than 95 minutes.

Lonsdale and Sutor (1971) demonstrated that the solid

portion of the bird urine consists of irregular layers of uric acid dihydrate and trapped soluble materials. McNabb et al. (1973) reported between 40 and 75 percent (depending on dietary protein level) and 20 percent of the total excreted sodium and potassium, respectively, was contained in the precipitated fraction of urine in the rooster. It was found that less sodium and more potassium (depending on treatment) were excreted (percentagewise) in the precipitated urine in Experiment 2 (Table 26) than was observed by McNabb. The most probable explanation for the lower percentage of sodium is that the dilution of the urine with deionized distilled water at the time of collection prevented the sodium from being trapped in the soluble layers between layers of uric acid crystals as McNabb and his collaborators have suggested. The data indicates that significantly less sodium and potassium, percentagewise, were excreted in the solid phase of the urine after corticosterone treatment (Table 26). From the data of McNabb et al.(1973) there appears to be an inverse relationship between the liquid phase of urine sodium and potassium concentration, and percentage excreted cation in the precipitate. This is contrary to results of Experiment 2 (Tables 19 and 26). The corticosterone treated birds had the lowest urine sodium and potassium concentrations and very low solid phase sodium and potassium excretion; the solid urine response to corticosterone treatment was highly variable but not significant. This would

occur if urine hydrogen ion (or ammonium ion) concentration increased. Urate has a higher affinity for these cations than for sodium or potasium (Porter 1963a, 1963b). Therefore, it is possible that corticosterone increased acid excretion.

TABLE 24

The Effects of Aldosterone Infusion Rate on the Clearance of Potassium for 95 Minutes C_K (µl/min/kg) Aldosterone*

		Minutes			
	N	0-30	30-50	55-75	75-95
Control	5	92±6	103±9	133±14	215±33
0.04 µg/min	4	252±53	214±40	325±39	262±35
0.08 µg/min	5	242±34[a**]	258±25[ab]	356±82[b]	284±53[ab]
0.12 µg/min	5	382±49[a]	328±19[ab]	250±34[b]	259±33[ab]
0.16 µg/min	5	158±15	150±18	177±30	133±14

*See footnote, Table 6.

**See footnote, Table 6.

TABLE 25

The Effects of Aldosterone Infusion Rate on the Percent of Filtered Potassium Excreted for 95 Minutes
% E_K Aldosterone*

		Minutes			
	N	0-30	30-50	55-75	75-95
control	5	6.9±0.6	7.6±0.9	9.5±1.2	14.0±2.2
0.04 µg/min	4	18.5±3.5	13.6±2.5	20.4±2.7	17.2±2.2
0.08 µg/min	5	17.2±2.2	18.5±1.9	24.3±4.5	19.9±3.2
0.12 µg/min	5	26.6±2.4	25.1±1.7	19.7±2.3	20.6±2.6
0.16 µg/min	5	11.7±0.9	11.5±1.2	14.5±2.9	9.7±0.8

*See footnote, Table 6.

TABLE 26

The Effects of Aldosterone (0.35 mg/kg/day) and Corticosterone (14.0 mg/kg/day) Administered for 3 Days on Percent of Total Sodium and Potassium Excreted in Solid Urine*

	Control	Aldosterone	Corticosterone
	n = 4	n = 3	n = 3
Na	15.2±4.2^{a**}	11.1±3.5^{a}	3.7±2.3^{b}
K	39.2±7.8^{a}	24.5±9.1^{ab}	11.1±9.7^{b}

*See footnote, Table 8.

**Means within parameter with different superscripts are significantly different ($p < .01$).

CONCLUSIONS

1. Glomerular filtration rate (GFR) shows considerable variability between and within individual roosters.

2. Corticosterone at estimated physiological secretory rates can increase GFR at least transiently.

3. Aldosterone has no short term or long term effects on GFR.

4. Corticosterone can create a diuresis by increasing GFR or decreasing water resorption.

5. Aldosterone does not alter urine flow rate or water resorption.

6. Corticosterone has a short term naturetic effect which is attributable to increases in sodium filtration rates and decreases in sodium resorption.

7. Corticosterone, chronically administered, has an antinaturetic and kaluretic effect which is most probably associated with increased excretion of hydrogen and ammonium ions.

8. Aldosterone, chronically administered, has an antinaturetic effect but no kaluresis is observed.

9. Aldosterone can cause contrasting results,

depending on dose, up to 95 minutes after administration on potassium excretion, but sodium excretion is not affected during this time indicating that the latency period for aldosterone action may be longer than 95 minutes.

10. The data support the concept that sodium retention and potassium secretion are not linked in the distal tubule.

11. Liquid urine excretion rates of sodium and potassium reflect total urine excretory rates.

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APPENDIX

Clearance Data Experiment 1

TABLE 1

Corticosterone
GFR (ml/min/kg)*

Minutes	Control	3.0 μg/min	6.0 μg/min	9.0 μg/min	12.0 μg/min
0-10	1.36±.12 (5)*	1.74±.24 (4)	1.43±.08 (5)	2.08±.24 (4)	1.04±.23 (4)
10-20	1.37±.27	1.45±.10	1.43±.27	2.49±.23	1.25±.18
20-30	1.47±.19	1.46±.08	1.46±.23	2.25±.35	1.36±.21
30-40	1.39±.18	1.31±.25	1.47±.32	1.86±.25	1.35±.15
40-50	1.47±.12	1.85±.27	1.77±.38	2.39±.26	1.34±.22
55-65	1.43±.08	1.43±.09	1.83±.33	3.14±.37	1.41±.30
65-75	1.49±.14	1.49±.14	1.94±.26	2.96±.33	1.38±.28
75-85	1.60±.17	1.20±.17	1.68±.20	2.44±.16	1.44±.28
85-95	1.62±.14	1.51±.16	1.51±.31	2.91±.24	1.45±.32
P	NS	NS	$<.10$	$<.05$	NS

*Mean ± Standard Error (n).

TABLE 2

Corticosterone
V (µl/min/kg)*

Minutes	Control	3.0 µg/min	6.0 µg/min	9.0 µg/min	12.0 µg/min
0-10	29.6±10.6 (5)*	19.2±9.9 (4)	14.0±1.8 (5)	17.2±3.1 (4)	31.4±19.9 (4)
10-20	23.4±6.7	21.4±8.3	13.9±2.0	19.2±4.1	28.8±16.2
20-30	23.9±8.7	19.4±6.4	14.1±3.2	18.7±4.1	27.1±13.3
30-40	22.3±8.2	20.2±6.4	13.6±2.3	20.1±4.6	41.4±16.3
40-50	19.9±7.3	19.7±3.5	13.6±2.5	21.5±4.8	73.8±35.0
55-65	23.6±6.3	18.8±2.8	15.8±3.3	41.2±15.5	81.4±46.1
65-75	25.5±8.0	26.6±4.6	18.0±1.8	42.7±21.2	81.4±46.4
75-85	24.0±8.0	26.3±2.8	18.3±1.9	42.4±19.9	69.3±45.1
85-95	23.7±7.2	22.8±2.6	16.6±3.1	34.2±13.1	53.6±36.1
P	NS	NS	NS	$<.05$	$<.05$

*See Table 1.

TABLE 3

Corticosterone
Reab H_2O (%)*

Minutes	Control	3.0 µg/min	6.0 µg/min	9.0 µg/min	12.0 µg/min
1-10	97.63 ±.96 (5)*	98.68 ±.82 (4)	99.02 ±.13 (5)	99.16 ±.14 (4)	96.94 ±1.74 (4)
10-20	98.11 ±.63	98.39 ±.75	98.98 ±.14	99.22 ±.17	97.87 ±1.03
20-30	98.27 ±.65	98.61 ±.53	99.06 ±.15	99.18 ±.10	98.09 ±.85
30-40	98.36 ±.60	98.46 ±.41	99.02 ±.14	98.95 ±.15	97.09 ±1.04
40-50	98.56 ±.56	98.82 ±.32	99.19 ±.13	99.06 ±.24	94.72 ±2.18
55-65	98.31 ±.46	98.70 ±.15	99.15 ±.13	98.65 ±.47	94.46 ±2.16
65-75	98.16 ±.61	98.07 ±.55	99.01 ±.15	98.41 ±.87	94.75 ±2.32
75-85	98.16 ±.61	97.66 ±.38	98.88 ±.11	98.31 ±.72	96.47 ±2.05
85-95	98.38 ±.61	98.44 ±.21	98.80 ±.28	98.83 ±.42	96.74 ±1.69
P	NS	NS	NS	NS	<.05

*See Table 1.

TABLE 4

Corticosterone
E_{Na} (μeq/min/kg)*

Minutes	Control	3.0 μg/min	6.0 μg/min	9.0 μg/min	12.0 μg/min
0-10	1.85±.49 (5)*	0.82±.11 (4)	1.70±.36 (5)	1.52±.47 (4)	1.95±.83 (4)
10-20	1.40±.34	0.69±.15	1.50±.31	1.47±.51	1.86±.71
20-30	1.56±.31	0.78±.19	1.46±.31	1.13±.33	1.94±.91
30-40	1.33±.23	0.92±.37	1.47±.14	1.56±.55	1.76±.46
40-50	1.32±.50	1.13±.39	1.77±.49	1.60±.64	1.76±.57
55-65	1.35±.17	1.57±.51	2.65±.52	2.08±.52	1.89±.73
65-75	1.54±.17	1.95±.59	1.96±.29	2.40±.72	1.62±.70
75-85	1.82±.36	1.57±.56	2.20±.42	1.29±.37	1.67±.77
85-95	2.12±.34	1.76±.75	1.94±.36	1.31±.38	1.58±.69
P	NS	$<.05$	$<.05$	$<.05$	NS

*See Table 1.

TABLE 5

Corticosterone
$\%E_{Na}$*

Minutes	Control	3.0 μg/min	6.0 μg/min	9.0 μg/min	12.0 μg/min
0-10	0.84±.16 (5)*	0.34±.07 (4)	0.77±.16 (5)	0.53±.15 (4)	1.05±.40 (4)
10-20	0.63±.06	0.32±.08	0.73±.14	0.43±.15	0.86±.25
20-30	0.66±.08	0.34±.06	0.70±.13	0.34±.07	0.84±.34
30-40	0.61±.09	0.46±.15	0.74±.11	0.58±.19	0.80±.20
40-50	0.54±.16	0.45±.18	0.71±.17	0.53±.22	0.76±.14
55-65	0.59±.07	0.71±.20	0.97±.12	0.53±.17	0.78±.19
65-75	0.68±.11	0.91±.29	0.69±.10	0.63±.19	0.66±.17
75-85	0.76±.20	0.86±.28	0.86±.13	0.39±.12	0.61±.18
85-95	0.90±.22	0.78±.29	0.87±.07	0.34±.11	0.62±.17
P	NS	$<.05$	NS	NS	NS

*See Table 1.

TABLE 6

Corticosterone
C_{Na} (µl/min/kg)*

Minutes	Control	3.0 µg/min	6.0 µg/min	9.0 µg/min	12.0 µg/min
0-10	115±27 (5)*	54±07 (4)	113±25 (5)	109±34 (4)	116±49 (4)
10-20	87±19	46±10	101±23	106±38	114±42
20-30	98±18	51±12	97±21	82±25	116±53
30-40	84±13	50±22	98±12	113±43	105±27
40-50	82±28	73±23	120±35	117±49	105±33
55-65	85±10	101±30	177±37	150±40	113±43
65-75	97±11	126±36	131±20	175±56	97±41
75-85	115±23	101±34	147±30	93±28	100±46
85-95	135±23	113±45	130±27	95±29	95±41
P	NS	$<.05$	$<.05$	$<.05$	NS

*See Table 1.

TABLE 7

Corticosterone
EK (μeq/min/kg)*

Minutes	Control	3.0 μg/min	6.0 μg/min	9.0 μg/min	12.0 μg/min
0-10	0.35±.04 (5)*	1.09±.35 (4)	1.17±.56 (5)	1.13±.62 (4)	0.60±.10 (4)
10-20	0.28±.04	0.99±.33	0.84±.12	1.07±.46	0.72±.14
20-30	0.39±.05	1.12±.35	0.93±.15	0.99±.44	0.71±.17
30-40	0.40±.06	0.95±.31	0.86±.07	1.32±.54	0.80±.16
40-50	0.36±.07	0.99±.31	0.91±.18	1.31±.48	0.76±.12
55-65	0.45±.06	0.93±.36	1.07±.27	1.58±.61	0.80±.07
65-75	0.62±.12	0.92±.25	1.45±.30	1.47±.47	0.82±.11
75-85	0.74±.19	0.72±.22	1.49±.19	1.13±.52	0.84±.18
85-95	0.91±.20	0.82±.24	1.36±.14	1.43±.72	0.92±.22
P	NS	NS	NS	NS	NS

*See Table 1.

TABLE 8

Corticosterone
$\%E_K$ *

Minutes	Control	3.0 µg/min	6.0 µg/min	9.0 µg/min	12.0 µg/min
0-10	7.4±.9 (5)*	16.9±6.3 (4)	18.6±6.7 (5)	14.0±6.3 (4)	21.4±5.6 (4)
10-20	5.8±.7	16.5±5.0	18.2±4.4	11.0±3.4	21.3±6.2
20-30	7.6±1.2	18.5±5.8	20.1±4.3	11.7±3.7	19.0±5.1
30-40	8.5±1.5	19.3±5.2	18.6±3.7	19.0±5.5	18.9±3.9
40-50	6.7±1.1	15.9±5.9	16.8±4.6	15.9±5.9	21.1±6.2
55-65	8.2±1.2	15.4±4.5	16.2±4.2	14.0±4.7	20.1±4.7
65-75	10.9±2.1	14.1±2.1	20.2±3.9	14.6±5.1	19.9±4.1
75-85	13.8±4.0	13.5±3.9	23.5±3.9	11.0±4.0	18.4±3.4
85-95	14.2±2.5	12.0±2.8	24.7±4.7	11.7±4.9	20.8±5.2
P	NS	NS	NS	NS	NS

*See Table 1.

TABLE 9

Corticosterone
C_K (l/min/kg)*

Minutes	Control	3.0 µg/min	6.0 µg/min	9.0 µg/min	12.0 µg/min
0-10	96 ± 8 (5)*	270 ± 98 (4)	285 ± 119 (5)	324 ± 172 (4)	203 ± 40 (4)
10-20	76 ± 11	240 ± 79	231 ± 41	295 ± 117	236 ± 37
20-30	103 ± 13	273 ± 95	259 ± 44	270 ± 114	228 ± 37
30-40	112 ± 12	243 ± 79	231 ± 18	363 ± 140	245 ± 37
40-50	95 ± 12	257 ± 77	257 ± 58	370 ± 125	242 ± 30
55-65	115 ± 14	230 ± 81	258 ± 58	451 ± 164	247 ± 31
65-75	152 ± 24	215 ± 47	364 ± 65	415 ± 164	248 ± 43
75-85	199 ± 49	166 ± 46	370 ± 46	282 ± 113	256 ± 68
85-95	231 ± 50	191 ± 56	316 ± 57	357 ± 161	276 ± 85
P	NS	NS	NS	NS	NS

*See Table 1.

TABLE 10

Corticosterone
Filtered K (eg/min/kg)*

Minutes	Control	3.0 µg/min	6.0 µg/min	9.0 µg/min	12.0 µg/min
0-10	4.87±.56 (5)*	7.25±1.00 (4)	5.50±.67 (5)	7.11±.94 (4)	3.09±.61 (4)
10-20	5.03±1.11	5.94 .40	5.20±.84	8.72±1.09	3.78±.65
20-30	5.74±1.19	6.04 .45	5.33±.91	7.99±1.33	4.14±.72
30-40	4.97±.85	5.19 1.03	5.37±.99	6.5±1.00	4.40±.74
40-50	5.47±.55	7.08 .99	6.27±1.09	8.22±1.06	4.11±.63
55-65	5.59±.42	5.61 1.10	7.18±.95	10.77± 1.39	4.55±.85
65-75	5.96±.73	6.27 .83	7.55±.98	10.24± 1.17	4.60±.84
75-85	5.90±.80	5.10 .91	6.67±.60	9.20±1.06	4.81±.82
85-95	6.22±.51	6.39 .79	6.25±1.00	10.92± 1.25	5.04±1.08

*See Table 1.

Clearance Data Experiment 2

TABLE 11

Aldosterone
GFR (ml/min/kg)*

Minutes	Control	0.04 μg/min	0.08 μg/min	0.12 μg/min	0.16 μg/min
0-10	1.36±.12 (5)*	1.24±.14 (4)	1.54±.17 (5)	1.54±.12 (5)	1.34±.09 (5)
10-20	1.37±.27	1.42±.31	1.44±.16	1.42±.15	1.40±.13
20-30	1.47±.19	1.40±.25	1.33±.15	1.28±.09	1.33±.15
30-40	1.39±.18	1.94±.41	1.60±.20	1.30±.13	1.34±.11
40-50	1.47±.12	1.42±.18	1.56±.37	1.38±.07	1.34±.16
55-65	1.43±.08	1.79±.13	1.47±.15	1.41±.34	1.28±.09
65-75	1.49±.14	1.51±.20	1.50±.29	1.54±.42	1.22±.10
75-85	1.60±.17	1.51±.11	1.32±.21	1.33±.07	1.27±.12
85-95	1.62±.14	1.55±.17	1.43±.17	1.22±.15	1.44±.04
P	NS	NS	NS	NS	NS

*See Table 1.

TABLE 12

Aldosterone
V (μl/min/kg)*

Minutes	Control	0.04 μg/min	0.08 μg/min	0.12 μg/min	0.16 μg/min
0-10	29.6 ± 10.6 (5)*	26.6 ± 12.5 (4)	29.8 ± 8.6 (5)	23.1 ± 5.0 (5)	16.8 ± 2.4 (5)
10-20	23.4 ± 6.7	27.1 ± 13.6	28.7 ± 5.9	18.0 ± 3.1	17.1 ± 1.6
20-30	23.9 ± 8.7	31.1 ± 16.0	23.9 ± 4.8	17.2 ± 2.9	16.7 ± 3.2
30-40	22.3 ± 8.2	32.5 ± 15.6	28.6 ± 6.9	17.8 ± 2.6	15.9 ± 2.1
40-50	19.9 ± 7.3	34.2 ± 16.8	28.4 ± 7.9	17.0 ± 3.1	17.7 ± 1.1
55-65	23.6 ± 6.3	31.6 ± 14.8	35.4 ± 11.4	19.2 ± 2.6	17.0 ± 3.5
65-75	25.5 ± 8.0	35.0 ± 13.5	31.3 ± 9.5	18.8 ± 3.0	21.3 ± 6.1
75-85	24.0 ± 7.2	37.3 ± 15.8	29.6 ± 6.0	22.6 ± 3.6	23.3 ± 6.8
85-95	23.7 ± 7.2	41.3 ± 15.5	34.4 ± 8.6	23.4 ± 4.4	24.9 ± 5.4
P	NS	NS	NS	NS	NS

*See Table 1.

TABLE 13

Aldosterone
Reab H_2O (%)*

Minutes	Control	0.04 μg/min	0.08 μg/min	0.12 μg/min	0.16 μg/min
0-10	97.63±.96 (5)*	98.03±.70 (4)	97.76±.82 (5)	98.56±.20 (5)	98.76±.15 (5)
10-20	98.11±.63	98.33±.45	97.72±.70	98.73±.16	98.74±.18
20-30	98.27±.65	98.17±.62	98.12±.36	98.67±.17	98.79±.11
30-40	98.36±.60	98.52±.38	98.17±.41	98.59±.21	98.81±.12
40-50	98.56±.56	97.78±.76	97.62±.91	98.79±.17	98.58±.26
55-65	98.31±.46	98.31±.72	97.22±1.17	97.87±.96	98.70±.25
65-75	98.16±.61	97.88±.53	97.22±1.17	98.60±.29	98.13±.64
75-85	98.32±.59	97.71±.79	97.20±1.50	98.24±.36	98.25±.50
85-95	98.38±.61	97.53±.66	97.15±1.15	97.99±.42	98.24±.42
P	NS	NS	NS	NS	NS

*See Table 1.

TABLE 14

Aldosterone
E_{Na} (μeq/min/kg)*

Minutes	Control	0.04 μg/min	0.08 μg/min	0.12 μg/min	0.16 μg/min
0-10	1.85±.49 (5)*	2.00±.99 (4)	3.81±2.05 (5)	0.85±.09 (5)	1.48±.38 (5)
10-20	1.40±.34	2.72±1.48	2.84±6.17	0.91±.41	1.50±.27
20-30	1.76±.31	1.82±1.01	2.09±.69	0.64±.28	1.63±.40
30-40	1.33±.23	1.73±.82	3.11±1.32	0.86±.29	1.74±.20
40-50	1.32±.50	2.37±1.36	2.64±.94	0.87±.27	1.52±.23
55-65	1.35±.17	2.68±1.48	3.04±1.02	1.00±.29	1.40±.18
65-75	1.54±.17	3.21±1.38	2.49±1.03	0.74±.16	1.99±.46
75-85	1.82±.36	2.75±1.24	3.15±1.63	0.99±.26	1.29±.31
85-95	2.12±.34	2.80±1.22	2.44±.88	0.80±.20	1.33±.24
P	NS	NS	NS	NS	NS

*See Table 1.

TABLE 15

Aldosterone
$\%E_{Na}$*

Minutes	Control	0.04 μg/min	0.08 μg/min	0.12 μg/min	0.16 μg/min
0-10	0.84±.16 (5)*	1.11±.40 (4)	1.91±1.00 (5)	0.34±.04 (5)	0.74±.21 (5)
10-20	0.63±.06	1.27±.45	1.59±.71	0.37±.15	0.77±.22
20-30	0.66±.08	0.77±.29	1.22±.47	0.29±.12	0.81±.15
30-40	0.61±.09	0.65±.28	1.38±.59	0.38±.10	0.90±.14
40-50	0.54±.16	1.11±.51	1.50±.57	0.37±.10	0.79±.13
55-65	0.59±.07	1.05±.50	1.59±.54	0.73±.47	0.75±.12
65-75	0.68±.11	1.60±.77	1.34±.69	0.31±.05	1.14±.30
75-85	0.76±.20	1.34±.58	1.69±.67	0.44±.10	0.67±.13
85-95	0.90±.22	1.51±.84	1.31±.55	0.42±.14	0.62±.11
P	NS	NS	NS	NS	NS

*See Table 1.

TABLE 16

Aldosterone
C_{Na}(ml/min/kg)*

Minutes	Control	0.04 µg/min	0.08 µg/min	0.12 µg/min	0.16 µg/min
0-10	115±27 (5)*	148±66 (4)	247±119 (5)	51±6 (5)	99±26 (5)
10-20	87±19	199±96	188±66	55±25	100±19
20-30	98±18	134±66	137±38	39±17	109±27
30-40	84±13	136±63	203±78	52±18	116±13
40-50	82±28	181±93	174±52	52±17	102±16
55-65	85±10	204±101	207±53	60±18	94±13
65-75	97±11	263±125	163±60	44±10	132±30
75-85	115±23	217±98	208±99	59±15	86±22
85-95	135±23	240±126	162±48	48±12	80±16
P	NS	NS	NS	NS	NS

*See Table 1.

TABLE 17

Aldosterone
E_K (μeq/min/kg) *

Minutes	Control	0.04 μg/min	0.08 μg/min	0.12 μg/min	0.16 μg/min
0-10	0.35±.04 (5)*	0.93±.32 (4)	0.82±.08 (5)	1.72±.28 (5)	0.69±.15 (5)
10-20	0.28±.04	1.26±.50	0.85±.16	1.52±.30	0.64±.14
20-30	0.39±.05	0.93±.28	0.65±.10	1.10±.16	0.60±.15
30-40	0.40±.06	0.80±.20	0.81±.11	1.16±.12	0.68±.15
40-50	0.36±.07	0.98±.31	0.71±.04	1.31±.11	0.54±.10
55-65	0.45±.06	1.29±.37	1.41±.42	1.00±.19	0.64±.13
65-75	0.62±.12	1.46±.33	0.96±.22	0.93±.16	0.78±.24
75-85	0.74±.19	1.26±.32	1.14±.23	1.22±.30	0.51±.11
85-95	0.91±.20	1.11±.23	1.06±.36	0.91±.16	0.55±.04
P	$<.10$	NS	$<.05$	$<.05$	NS

*See Table 1.

TABLE 18

Aldosterone
$\%E_K$ *

Minutes	Control	0.04 μg/min	0.08 μg/min	0.12 μg/min	0.16 μg/min
0-10	7.37±.93	18.40± 5.10	17.15± 2.93	32.01± 4.09	13.26± 1.85
	(5)*	(4)	(5)	(5)	(5)
10-20	5.81±.65	23.40± 9.03	19.71± 5.09	27.18± 4.16	10.94± 1.66
20-30	7.63± 1.24	13.83± 3.41	14.60± 3.55	20.55± 3.09	10.94± 1.48
30-40	8.50± 1.53	10.79± 3.41	19.01± 1.45	24.50± 3.13	11.9 ± 1.19
40-50	6.70± 1.12	16.47± 3.35	17.98± 3.67	25.68± 1.81	11.04± 2.27
55-65	8.16± 1.21	18.66± 3.99	27.74± 7.03	19.67± 2.87	11.89± 1.32
65-75	10.86± 2.12	22.23± 3.97	20.87± 5.85	19.78± 4.00	17.05± 5.67
75-85	13.77± 4.03	18.27± 3.05	22.12± 3.14	20.88± 4.39	9.79± 1.43
85-95	14.17± 2.54	16.14± 2.52	16.63± 5.47	20.39± 3.18	9.63± 0.90
P	NS	NS	NS	NS	NS

*See Table 1.

TABLE 19

Aldosterone
C_K (µl/min/kg)*

Minutes	Control	0.04 µg/min	0.08 µg/min	0.12 µg/min	0.16 µg/min
0-10	96.3 ± 8.0 (5)*	230.5 ± 76.4 (4)	249.7 ± 38.8 (5)	511.0 ± 103.7 (5)	174.9 ± 24.8 (5)
10-20	76.0 ± 10.7	325.5 ± 136.6	275.5 ± 82.1	372.8 ± 63.3	150.8 ± 26.6
20-30	102.6 ± 13.0	201.2 ± 58.2	200.3 ± 54.3	264.5 ± 48.9	148.8 ± 32.1
30-40	111.9 ± 14.0	188.3 ± 42.5	266.3 ± 30.9	306.9 ± 33.5	164.2 ± 30.1
40-50	94.5 ± 11.6	239.5 ± 72.1	250.0 ± 52.2	349.1 ± 14.7	135.2 ± 21.3
55-65	114.8 ± 13.7	324.6 ± 64.8	440.4 ± 146.8	255.4 ± 58.7	154.7 ± 25.3
65-75	151.9 ± 23.9	325.5 ± 55.4	271.7 ± 71.7	245.0 ± 42.7	198.8 ± 55.7
75-85	198.8 ± 48.5	275.1 ± 61.4	304.0 ± 71.6	278.2 ± 59.9	127.63 ± 264
85-95	230.8 ± 49.5	248.3 ± 41.5	260.8 ± 83.7	239.3 ± 31.7	138.2 ± 11.3
P	NS	NS	$<.05$	$<.05$	NS

*See Table 1.

TABLE 20

Aldosterone
Filtered K (μeq/min/kg) *

Minutes	Control	0.04 μg/min	0.08 μg/min	0.12 μg/min	0.16 μg/min
0-10	4.87±.56 (5)*	4.64±.59 (4)	5.17±.64 (5)	5.74±.29 (5)	5.23±.70 (5)
10-20	5.03±1.11	5.35±1.08	4.89±.75	5.99±1.04	6.01±.90
20-30	5.74±1.19	6.46±1.03	4.73±.67	5.39±.42	5.25±.81
30-40	4.97±.85	7.92±1.91	4.22±.38	5.06±.73	5.40±.70
40-50	5.47±.55	5.47±.87	4.61±.95	5.18±.42	5.12±.47
55-65	5.59±.42	6.45±.80	4.96±.37	5.56±1.18	5.15±.54
65-75	5.96±.73	6.41±.97	5.74±1.41	5.81±1.52	4.68±.43
75-85	5.90±.80	6.58±.60	5.03±.68	5.69±.47	4.97±.51
85-95	6.22±.51	6.76±.87	6.80±.73	4.73±.96	5.81±.44
P	NS	NS	NS	NS	NS

*See Table 1.