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# ALDOSTERONE EFFECTS ON RENAL METABOLISM

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SUMMARY

1. Manometric studies of the sodium dependent oxygen consumption in rat kidney slices have failed to reveal any significant effect of aldosterone treatment, adrenalectomy or sodium diet on sodium metabolism.

2. However, ammonia release from kidney slices was significantly reduced following adrenalectomy and this decrease was influenced by aldosterone treatment.

3. The dose–response characteristic obtained for this aldosterone-stimulated ammonia release has been determined.

4. The effect of a high $Na^+$ diet on the ammonia release has been studied. An initial decrease after 2 days may be associated with decreased endogenous aldosterone secretion. However, aldosterone (2·5 $\mu$g/100 g body weight) injections into these high $Na^+$ treated animals fails to restore the normal ammonia release.

5. The effects of aldosterone (2·5 $\mu$g/100 g body weight), dexamethasone (2·5 $\mu$g/100 g body weight) and corticosterone (2·5 $\mu$g/100 g body weight) injections, in adrenalectomized rats, on ammonia release and tissue tyrosine aminotransferase activities have been compared.

## INTRODUCTION

In studies of the mechanism of aldosterone action in the toad bladder (Leaf, 1965; Edelman, 1966) considerable attention has been given to the problem of which process normally limits sodium transport. Sharp & Leaf (1966) and Snart (1970) favour the idea that sodium transport across the bladder is normally limited by the permeability of the mucosal surface and that the effect of aldosterone is to increase this permeability. Edelman & Fanestil (1970) on the other hand support the view that the ion pump is normally rate limiting and that aldosterone acts to increase the activity of this enzyme pump, independent of any effect on mucosal permeability. Snart (1972) in an analysis of metabolic changes associated with aldosterone action in toad bladder proposed a two stage mechanism for the aldosterone response associated with the saturation of two types of aldosterone-receptors. Evidence was presented supporting the idea that the principal effect of aldosterone is to increase mucosal sodium permeability. However, a second effect of the hormone on key metabolic enzymes may allow for an increased supply of high energy intermediate to the pump, once the pump becomes rate limiting.

In the kidney, sodium uptake along the length of the tubule involves several different mechanisms, the details of which are being investigated by use of micro-

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puncture techniques (Burg & Green, 1973; Imai & Kokko, 1974; Windhager & Giebisch, 1976). Aldosterone affects total sodium uptake to a limited extent (Vander, 1975) and the cortical collecting tubule has been identified as a site for mineralocorticoid activity (Gross, Imai & Kokko, 1975). Although it is well known that both renal sodium uptake and potassium excretion are affected by aldosterone (Kirsten & Kirsten, 1972) there is evidence to suggest that this exchange is not coupled (Williamson, 1963). In the vertebrate kidney, sodium uptake is usually seen in relation to acid–base regulatory mechanisms whereby sodium uptake may be coupled to the secretion of hydrogen ions (Harper, 1973, Malnic & Steinmetz, 1976). In the proximal part of the tubule this proton exchange may proceed in the presence of bicarbonate and other buffering anions whereas in the distal part of the tubule the urine is no longer able to buffer secreted hydrogen ions and it is believed that in this region the $H^+$ secretion is accompanied by ammonia release, following deamination of amino acids (Harper, 1973).

Aldosterone is active in the distal part of the tubule and Crabbé and Nichols (1960) have shown that ammonia release from cortical slices, taken from adrenalectomized rats, may be stimulated by aldosterone. Kirsten & Kirsten (1972) and Kinne & Kirsten (1968) have shown that several cytoplasmic and mitochondrial enzymes are stimulated by aldosterone and that the redox state of the pyridine nucleotides is increased. In other studies Rousseau, Baxter, Funder, Edelman & Tomkins (1972), Funder, Feldman & Edelman (1973) have identified two types of receptor protein for aldosterone binding in rat kidney. The one with highest affinity for aldosterone was shown to be specific for the mineralocorticoid, whereas the second was shown to bind dexamethasone and corticosterone more tightly than aldosterone.

In the present work we have studied oxygen consumption changes in isolated kidney slices, under various conditions, in an attempt to realize any metabolic changes that may be associated with mineralocorticoid activity. Effects of aldosterone on transaminase activity and ammonia release have been studied in order to investigate the possible role of ammonia production and release in the mechanism of aldosterone action.

## METHODS

Male Wistar rats (180–220 g) maintained on an Oxoid 86 diet and tap water were used throughout this work unless otherwise stated. Before each experiment the rats were killed by a blow to the head, and the kidneys rapidly excised, decapsulated and sliced. Two slices were taken from each side of the kidney; the small amount of medulla was not removed.

### *Oxygen consumption in kidney slices*

Standard manometric techniques were used to study the oxygen consumption over a four hour period in individual kidney slices (0·5 mm thick), taken from control rats, incubated in the presence or absence of $10^{-7}$ M-aldosterone in Krebs phosphate Ringer solution at 37 °C. The effect of changing $Na^+$ concentration on this respiration was studied in a corresponding series of experiments in which sodium was replaced by choline. Similar studies were carried out on adrenalectomized animals (4 days after adrenalectomy) and on animals maintained on an Oxoid 86 diet with free access to 0·9% saline (1 year). The results have been expressed in terms of the mean value of the $Q_{O_2}$ ($\mu$l $O_2$ consumed/g dry tissue weight.hour) measured in several kidney slices.

*Ammonia released by kidney slices*

Six untreated rats were killed and the kidneys excised, decapsulated and sliced using a Stadie-Riggs microtome. Individual slices (0·5 mm thick) were transferred to small conical flasks containing 2 ml. Krebs phosphate Ringer solution and incubated at 37° for 15 min. Each kidney slice was removed and 200 $\mu$l. incubation medium neutralized with 1 ml. saturated potassium carbonate, shaken at room temperature for 30 min and 10 ml. Nessler solution added. The optical density at 420 nm was then measured against a control blank using a Unicam SP 500 spectrophotometer. A standard curve, obtained prior to each experiment, was used in order to express the results in terms of $\mu$mole ammonia/mg dry wt. tissue.hr. This experiment has been repeated using six adrenalectomized animals (4 days) and the effect of aldosterone was studied using further groups of six adrenalectomized animals (4 days) injected subcutaneously 4 hr before killing with 100 $\mu$l. 10% EtOH in 0·9% saline in the absence or presence of varying concentrations of aldosterone (0·2–20 $\mu$g/100 g body wt.). In other experiments similar procedures were used in adrenalectomized rats following injections containing corticosterone (2·5 $\mu$g/100 g body wt.) and dexamethasone (2·5 $\mu$g/100 g body wt.), 5 hr before killing.

In a final group of experiments, animals were maintained on a high $Na^+$ diet (Oxoid 86 and 0·9% saline) for a month. In one group four animals were killed at 24 hr intervals over the first 6 days and at day 30. In a corresponding group, four animals were injected with aldosterone (2·5 $\mu$g/100 g body wt.) 4 hr prior to killing at the same intervals of time. In each group the ammonia release in isolated kidney slices was studied as described previously.

*Tyrosine aminotransferase activity*

The tyrosine amino transferase activity was measured using the method described by Granner & Tomkins (1970) in kidney samples taken from six adrenalectomized animals (4 days) that had been injected with aldosterone (2·5 $\mu$g/100 g body wt.), corticosterone (2·5 $\mu$g/100 g body wt.) or with 100 $\mu$l. 10% EtOH in 0·9% saline 5 hr before killing. Each measurement was repeated ten times on a pooled kidney homogenate obtained from four animals. Protein estimation was carried out using standard procedures and the results expressed in terms of $\mu$mole *p*-OH phenylpyruvate/g protein.min.

## RESULTS

Oxygen consumption measured in kidney slices taken from untreated animals was found to decrease very gradually over the 4 hr period. The effect of altering $Na^+$ concentration in the incubation medium (Fig. 1) indicates that after 4 hr the tissue respiration reflects the changing medium $Na^+$ concentration. The large medium dependent respiration has a $K_m$ for sodium of approximately 25 m-equiv./l., a value close to that found for the ($Na^+/K^+$)ATPase activity in the kidney (Jorgensen, 1972). The effect of adrenalectomy was investigated but no significant changes were observed: the $Q_{O_2}$ value for adrenalectomized rat kidney slices, measured under conditions comparable to those used with the nonadrenalectomized group included in Fig. 1, was $7{\cdot}31 \pm 0{\cdot}36$ ($n = 6$) in Krebs Ringer containing 154 m-equiv./l. $Na^+$. More extensive measurement of oxygen consumption in kidney slices from control rats gave a $Q_{O_2}$ value of $6{\cdot}87 \pm 0{\cdot}24$ ($n = 84$) after 4 hr incubation in Krebs Ringer solution and a value of $6{\cdot}76 \pm 0{\cdot}26$ ($n = 40$) in the presence of $10^{-7}$ M-aldosterone. The oxygen consumption in kidney slices taken from animals on a high $Na^+$ diet (a treatment designed to reduce endogenous aldosterone release) was also studied in Krebs Ringer solution. No significant difference in respiration rate was observed, with a $Q_{O_2}$ value of $6{\cdot}51 \pm 0{\cdot}49$ ($n = 6$), a result that is taken to reflect the limited role of the mineralocorticoid in active $Na^+$ uptake. As this technique failed to reveal any clear effects of mineralocorticoid activity on tissue respiration no further studies were carried out.

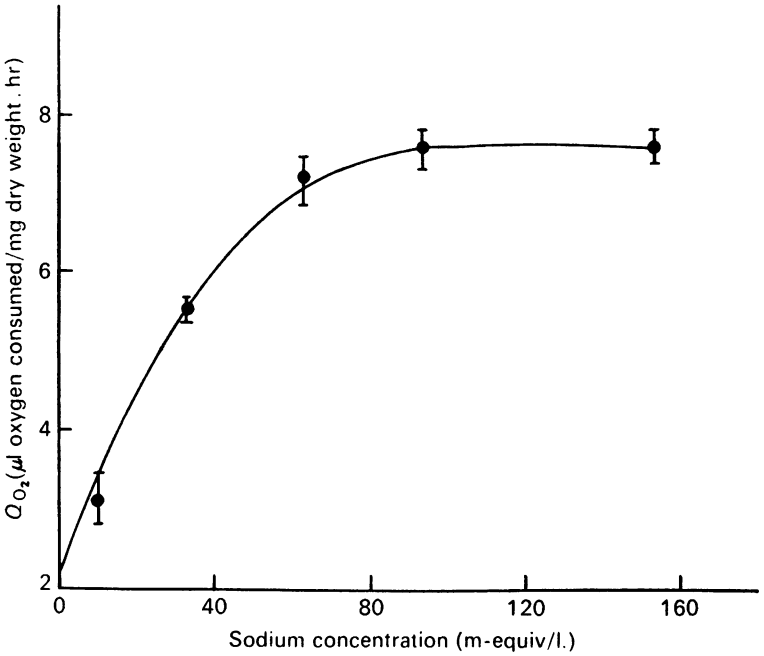


Fig. 1. Shows the mean $Q_{O_2}$ ($\mu$l. $O_2$ consumed/mg weight.hr) ± S.E. of mean measured in groups of ten kidney slices following 4 hr incubation in either Krebs Ringer solution (154 m-equiv/l. $Na^+$) or in Ringer of varying sodium concentrations, obtained by replacing sodium by choline. The 4 hr were required to allow for equilibration with the external medium.

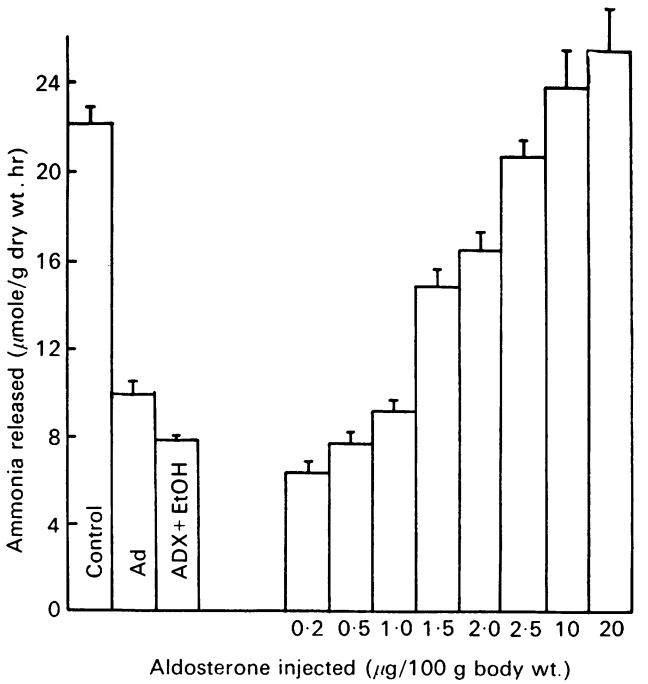


Fig. 2. The rate of ammonia release (mean ± S.E. of mean) from thirty-two or forty-eight kidney slices taken from groups of four or six normal or adrenalectomized (Ad) rats injected with 100 $\mu$l. 10% EtOH in 0·9% saline in the absence or presence of aldosterone (0·2–20 $\mu$g/100 g body wt.). The kidney slices were incubated in Krebs Ringer (2 ml.) for 15 min at 37 °C. The results have been expressed in terms of ammonia released per 200 $\mu$l. incubation medium.

In kidney slices taken from untreated animals the ammonia release measured over a 15 min interval was found to be $22{\cdot}1 \pm 0{\cdot}8$ $\mu$mole ammonia/g dry wt. tissue.hr. This rate of production was halved if taken over a full hour period. Adrenalectomy resulted in a large, significant ($P < 0{\cdot}001$) decrease in ammonia release (Fig. 2) in the kidney slices and this effect was shown to be reversed by aldosterone treatment. There was a significant response ($0{\cdot}02 > P > 0{\cdot}01$) to $1{\cdot}0$ $\mu$g aldosterone/100 g body wt. but a highly significant ($P < 0{\cdot}001$) increasing effect with doses of $1{\cdot}5$ $\mu$g/100 g body wt. and above. The dose response characteristic for the effect occurs over two orders of magnitude concentration range, a result that would be expected if the response were associated with the saturation of a hormone receptor.

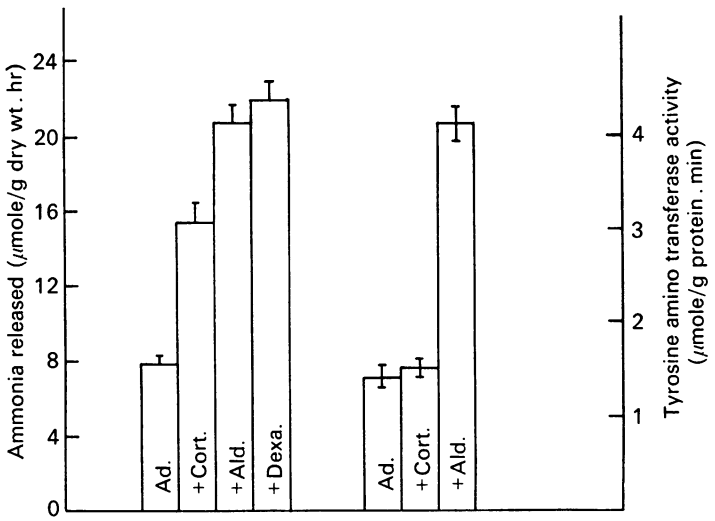


Fig. 3. Shows in histogram form the rate of ammonia release (mean ± S.E. of mean) from thirty-two kidney slices taken from groups of four adrenalectomized (Ad.) rats injected with 100 $\mu$l. 10% EtOH in 0·9% saline in the absence or presence of corticosterone (cort., 2·5 $\mu$g/100 g body wt.), aldosterone (Ald., 2·5 $\mu$g/100 g body wt.) or dexamethasone (Dexa., 2·5 $\mu$g/100 g body wt.). The effect of similar injections with or without corticosterone (2·5 $\mu$g/100 g body wt.) or aldosterone (2·5 $\mu$g/100 g body wt.) on renal tyrosine aminotransferase activity ($\mu$moles *p*-OH phenyl pyruvate/g protein.hr) is also shown (mean ± S.E. of mean for ten assays performed on pooled tissue from four rats in each group).

In further experiments, aldosterone (2·5 $\mu$g/100 g body wt.) caused a 167% increase in ammonia release from renal slices in adrenalectomized animals, while dexamethasone and corticosterone (2·5 $\mu$g/100 g body wt.) gave highly significant ($P < 0{\cdot}001$) increases of 180% and 97% respectively (Fig. 3). These results may be compared with the failure of similar doses of corticosterone to increase tyrosine amino transferase activity of the tissue (Fig. 3), but with the ability of aldosterone (2·5 $\mu$g/100 g body wt.) to stimulate this enzyme ($P < 0{\cdot}001$) (Fig. 3).

Results obtained using animals maintained on the high sodium diet show (Fig. 4) that the ammonia release in kidney slices was reduced to a minimum within 2 days after which it increased to reach normal values within 30 days. Injection of aldosterone (2·5 $\mu$g/100 g body wt.), 4 hr before killing, into corresponding groups of animals failed to restore the ammonia release to normal values in animals subjected to 2 days on a high $Na^+$ diet.

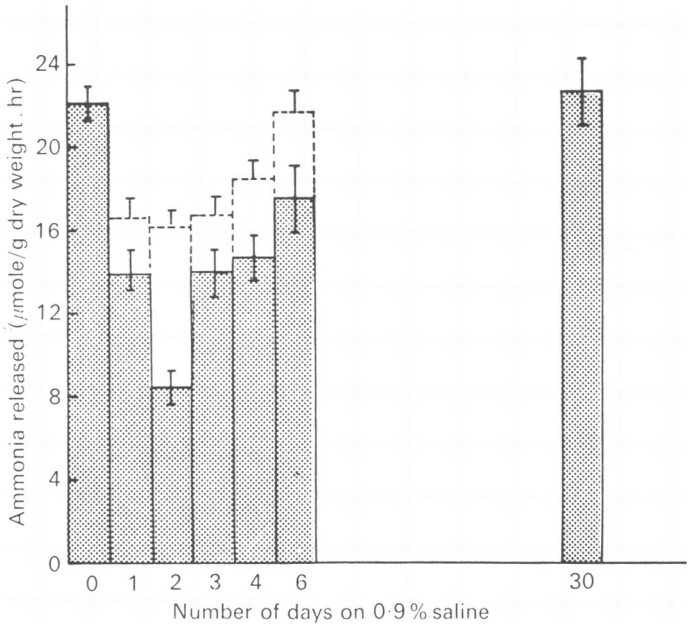


Fig. 4. The effect of a high $Na^+$ diet on the ammonia release from kidney slices (mean ± S.E. of mean of thirty-two measurements using four animals in each group; shaded histograms. The effects of injection with aldosterone (2·5 μg/100 g body wt.) 5 hr before killing, in corresponding groups of saline treated animals, are shown by the unshaded histograms.

## DISCUSSION

One of the most noticeable effects of aldosterone in the isolated toad bladder (Snart, 1972) is to increase oxygen consumption in the tissue after a 60–90 min lag period. The dose–response characteristic for this response revealed effects of the hormone on tissue metabolism occurring prior to any increase in sodium transport. In the present work we have looked for similar effects of aldosterone using isolated kidney slices. However, under corresponding conditions we have failed to observe any significant effect of aldosterone on oxygen consumption, a result that is consistent with a limited role of aldosterone on total active $Na^+$ uptake by the kidney.

Our studies of ammonia release by isolated kidney slices confirms the result of Crabbé & Nichols (1960), demonstrating that adrenalectomy significantly decreases ammonia release. Kirsten & Kirsten (1972), however, have shown that the ammonia concentration in kidneys taken from adrenalectomized rats is far higher than that in kidneys taken from control rats. It is also well accepted that the tubular cells are likely to be more alkaline in adrenalectomized rats due to $K^+$ retention. In our *in vitro* system, the ammonia release from adrenalectomized rat kidney slices is therefore favoured both by a concentration and a pH gradient. The observed decrease would thus argue in favour of a more direct $Na^+/NH_4^+$ exchange carrier system.

After two days on a high $Na^+$ diet the ammonia release from isolated kidney slices decreases to the level found after adrenalectomy (cf. Figs. 3 and 4). This observation may be a reflexion of the effect of high $Na^+$ in decreasing the release of endogenous aldosterone. However, with rats maintained on a high $Na^+$ diet for longer periods of

time, the ammonia release from isolated kidney slices returns to control values, although circulating aldosterone concentrations are still presumed to be decreased. This suggests an adaptive response of the tubular cells allowing for a maintained ammonia release. The aldosterone responsiveness of the renal ammonia release was significantly inhibited after 2 days on a high $Na^+$ diet and normal ammonia release could not be restored. This limitation on the aldosterone response was also evident in animals maintained for longer periods on the high $Na^+$ diet and ammonia release could not be stimulated above control values.

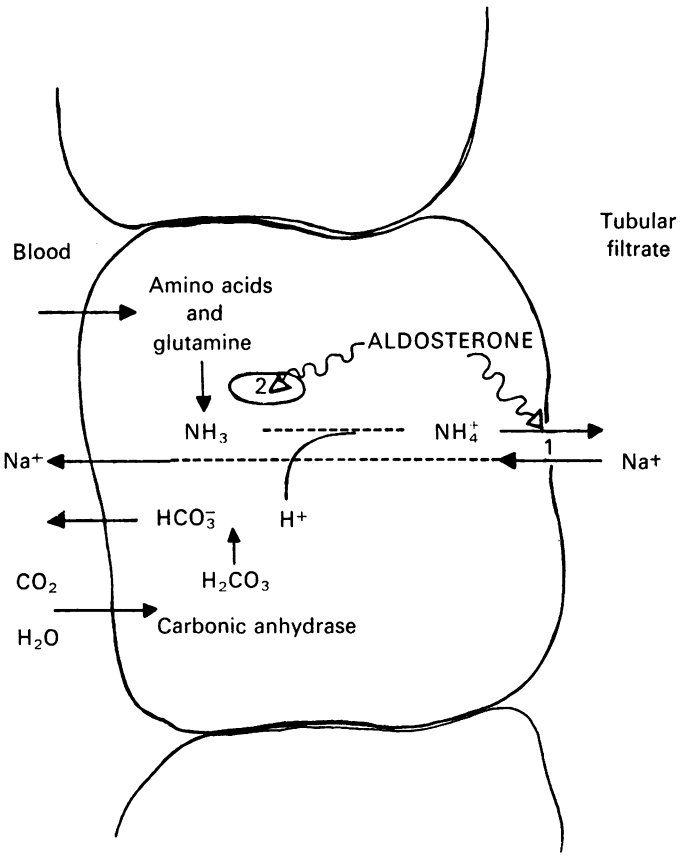


Fig. 5. Illustration of the proposed two stage nature of the aldosterone action in rat kidney. The major effect of the hormone is to increase sodium permeability at the mucosal surface (1). The second effect (2) on certain key mitochondrial enzymes including transaminase enzymes, increases the redox state of the tissue allowing for increased activity of the sodium pump (Kirsten & Kirsten, 1972) and for an increase in ammonia production and release.

In our final experiments we compared the glucocorticoid and mineralocorticoid effects on ammonia release from kidney slices. The results indicate that dexamethasone and corticosterone both affect this ammonia release, more or less, as effectively as aldosterone. We therefore believe that this response may be associated with the less specific mineralocorticoid/glucocorticoid receptor identified by Funder, Feldman & Edelman (1973). However, we have found that aldosterone, but not corticosterone, is able to stimulate tyrosine amino transferase activity in the rat kidney. This

response may therefore be associated with the specific mineralocorticoid receptor identified by Funder *et al.* (1973).

In toad bladder the tightest binding receptor for aldosterone has ben associated (Snart, 1972) with the activation of certain key metabolic enzymes, whereas the second receptor has been associated with a sodium 'permease' effect. In rat kidney there appears to be a response of a key metabolic enzyme associated with the receptor having highest affinity for aldosterone and a second 'permease' effect that may be associated with a second receptor site having lower affinity for aldosterone. We conclude that our results provide some evidence of a two stage mechanism of aldosterone action in the rat kidney (Fig. 5) and that there are interesting parallels between the action of aldosterone in rat kidney and toad bladder.

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## REFERENCES

BURG, M. B. & GREEN, N. (1973). Functions of the thick ascending limbs of Henle's loop. *Am. J. Physiol.* **224**, 659–668.

CRABBÉ, J. & NICHOLS, G. (1960). Effect of adrenalectomy, aldosterone and dehydration on electrolyte metabolism of rat renal cortex slices. *Am. J. Physiol.* **199**, 871–875.

EDELMAN, I. S. (1966). Mode of action of aldosterone. In *Steroid Dynamics*, ed. PINCUS, G., NAKAO, T. & TAIT, J. F., pp. 551–565. New York: Academic.

EDELMAN, I. S. & FANESTIL, D. D. (1970). *Mineralocorticoids in Biochemical Actions of Hormones*, ed. LITWACK, G., pp. 321–364. New York: Academic.

FUNDER, J. W., FELDMAN, D. & EDELMAN, I. S. (1973). Glucocorticoid receptors in rat kidney: the binding of tritiated dexamethasone. *Endocrinology* **92**, 1005–1013.

GRANNER, D. K. & TOMKINS, G. M. (1970). Tyrosine aminotransferase (rat liver). In *Methods in Enzymology*, vol. 5, ed. TABER, H. & TABOR, C. W., pp. 633–637. New York: Academic.

GROSS, J. B., IMAI, M. & KOKKO, J. P. (1975). A functional comparison of the cortical collecting tubule and the distal convoluted tubule. *J. clin. Invest.* **55**, 1284–1294.

HARPER, H. A. (1973). *Review of Physiological Chemistry*, 14th ed., p. 394. Los Altos, California: Lange Medical Publications.

IMAI, M. & KOKKO, J. P. (1974). Sodium chloride, urea and water transport in the thin ascending limb of Henle. *J. clin. Invest.* **53**, 393–402.

JORGENSEN, P. L. (1972). The role of aldosterone in the regulation of ($Na^+$ and $K^+$)ATPase in rat kidney. *J. Ster. Biochem.* **3**, 181–191.

KINNE, R. & KIRSTEN, R. (1968). Der einfluss von aldosterone auf die Aktivitat mitochondrialer und cytoplasmatischer enzyme in der Rattenniere. *Pfluges. Arch.* **300**, 244–254.

KIRSTEN, R. & KIRSTEN, E. (1972). Redox state of pyridine nucleotides in renal response to aldosterone. *Am. J. Physiol.* **223**, 229–235.

LEAF, A. (1965). Transepithelial transport and its hormonal control in toad bladder. *Ergebn. Physiol.* **56**, 216-263.

MALNIC, G. & STEINMETZ, P. R. (1976). Transport processes in urinary acidification. *Kidney Int.* **9**, 172–188.

ROUSSEAU, G., BAXTER, J. D., FUNDER, J. W., EDELMAN, I. S. & TOMKINS, G. M. (1972). Glucocorticoid and mineralocorticoid receptors for aldosterone. *J. Ster. Biochem.* **3**, 219–227.

SHARP, G. W. G. & LEAF, A. (1966). Mechanism of action of aldosterone. *Physiol. Rev.* **46**, 593–633.

SNART, R. S. (1970). Mechanism of hormonal action. *Hormones* **1**, 233–256.

SNART, R. S. (1972). The two stage nature of the aldosterone response. *J. Ster. Biochem.* **3**, 129–136.

VANDER, A. J. (1975). *Renal Physiology*, p. 69. New York: McGraw-Hill.

WILLIAMSON, H. E. (1963). Mechanism of the antinatriuretic action of aldosterone. *Biochem. Pharmac.* **12**, 1449–1450.

WINDHAGER, E. E. & GIEBISCH, G. (1976). Proximal sodium and fluid transport. *Kidney Int.* **9**, 121–133.