Journal of Steroid Biochemistry, 1972, Vol. 3, pp. 151–159. Pergamon Press. Printed in Great Britain

EFFECT OF ALDOSTERONE ON SODIUM AND POTASSIUM TRANSPORT IN THE KIDNEY

M. WIEDERHOLT, C. BEHN, W. SCHOORMANS and L. HANSEN
Institut für Klinische Physiologie, Klinikum Steglitz der Freien Universität Berlin, 1 Berlin 45, Germany

SUMMARY

(1) In adrenalectomized rats dietary potassium (and sodium) intake modifies the effect of aldosterone on electrolyte excretion. Speculations on mode and tubular site of action of aldosterone are misleading, if urinary excretion rates are the only data available.

(2) The main site of action of aldosterone on potassium transport is located at the distal tubular level. The distal tubule and the collecting duct are mainly responsible for changes in the excretion pattern of potassium.

(3) With electrophysiological methods it can be demonstrated that the transport number for potassium in the distal tubule of adrenalectomized rats is reduced significantly and is normalized by acute administration of aldosterone (2 μg/100 gm b.w.). The data indicate that the relative permeability of the luminal membrane of the distal tubule is under the influence of mineralocorticosteroids. The impairment of distal potassium secretion in adrenal insufficiency is mainly due to a reduced permeability of the luminal membrane. Aldosterone stimulates potassium secretion by increasing the luminal permeability of the distal tubule.

SITE and mode of action of aldosterone on electrolyte transport along the nephron is still a controversial matter. Micropuncture experiments in the rat kidney [1–3] revealed that aldosterone stimulates sodium reabsorption in the distal as well as in the proximal tubule, in spite of striking differences in functional organisation between these segments. Dexamethasone, a steroid with predominant glucocorticoid activity, had no effect on sodium transport [3, 4] in a dose which normalized the impaired capacity of adrenalectomized rats to excrete a water load. With regard to the mechanism of action of aldosterone on sodium transport, micropuncture experiments were performed in rats treated with inhibitors of protein synthesis [3, 5]. Actinomycin D depressed proximal and distal sodium reabsorption in normal and in aldosterone substituted adrenalectomized animals. These data support the hypothesis that aldosterone acts via induction of protein synthesis [6, 7]. However, if we look upon the tubular epithelium as a pump-leak system, the data obtained in the proximal tubule of adrenalectomized rats cannot be explained solely by an inhibition of active sodium transport. It was postulated that passive backflux of sodium into the tubular lumen is also under the control of aldosterone [1].

In addition to effects on sodium reabsorption, aldosterone also modifies renal potassium transport. In adrenal insufficiency, potassium excretion is reduced and a marked retention of this ion ensues. After a time delay of about 1 h, aldosterone increases urinary loss of potassium whereas sodium excretion is reduced. The concept of an aldosterone dependent sodium–potassium exchange in the distal tubule has widely been accepted.

However, experimental data in the literature clearly indicate that the effects of aldosterone on excretion of sodium and potassium can frequently be dissociated. A kaliuresis may appear without concomitant antinatriuresis and *vice versa*

[8–10]. Finally, several groups have demonstrated that actinomycin D significantly inhibits the antinatriuretic but not the kaliuretic action of aldosterone [5, 7, 11–13].

To clarify the reasons for this dissociation between sodium and potassium excretion, clearance and micropuncture experiments have been performed in normal and adrenalectomized rats under varying dietetic conditions concerning potassium uptake. For description of methods see Hierholzer *et al.*[1] and Wiederholt *et al.*[3].

1. ALDOSTERONE AND THE INTERRELATION BETWEEN SODIUM AND POTASSIUM EXCRETION

Fimognari *et al.*[13] have demonstrated that dietary potassium intake modifies the effect of aldosterone on potassium excretion. In adrenalectomized rats on a stock diet potassium excretion was not influenced by aldosterone. On the other hand, in animals pretreated with low-potassium diet a distinct kaliuresis was observed after hormone administration.

Similar results were obtained in our laboratory. At varying time prior to the experiments, adrenalectomized rats were put on a low-potassium diet. In clearance experiments (Fig. 1) a diuresis was induced by infusion of sodium chloride and mannitol (2·5% mannitol in 0·9% NaCl. Prime dose of 7·5 ml, continuous infusion of 4·5 ml/h). The ensuing increase in sodium excretion could be reduced by acute intravenous administration of aldosterone (2 μg/100 g). The time delay of onset and the magnitude of the action of aldosterone is typical. Despite a clear antinatriuretic effect a kaliuresis in response to aldosterone failed to appear when basal excretion rates of potassium were high. After an 18–20 h period of dietary potassium deprivation, plasma potassium concentration was reduced from about 5 mM to a mean value of 3·6 mM. Only under these conditions could a consistent increase in potassium excretion be demonstrated after acute administration of aldosterone.

A further reduction of the plasma potassium concentration (Fig. 2) by a low potassium diet for 3 days did not change the antinatriuretic effect of aldosterone. On the other hand, the kaliuretic effect was completely abolished under conditions where potassium secretion is very low. These series of experiments clearly establish that dietary potassium (and sodium) intake modifies the effects of aldosterone on electrolyte excretion and that speculations on mode and tubular site of action of aldosterone are misleading if urinary excretion rates are the only data available.

2. TUBULAR SITE OF ACTION OF ALDOSTERONE ON POTASSIUM TRANSPORT

Micropuncture studies on mammalian nephrons indicate that the proximal tubule, the loop of Henle, the distal tubule and the collecting duct all possess the capacity for net reabsorption of potassium. Under the experimental conditions of a strong mannitol diuresis (Fig. 3) active transport of potassium (against an electrochemical gradient) can be demonstrated in the proximal tubule of the rat kidney. The transtubular concentration ratio for potassium (tubular fluid/plasma) in control rats is less than unity with a mean value of 0·82. A similar concentration ratio is demonstrable in adrenalectomized rats after administration of aldosterone (7·5 μg/100 g per day, over a period of 3 days). In both groups actinomycin D (200 μg/100 g) inhibited the potassium reabsorption in the proximal tubule. The

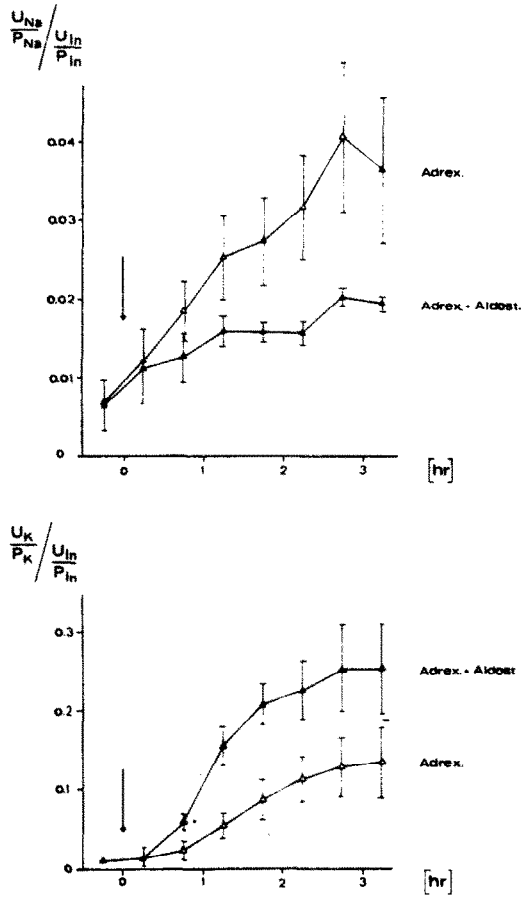

Fig. 1. Sodium and potassium excretion in adrenalectomized rats (180–220 g) on a low potassium diet for 18–20 h. Mean plasma potassium concentration 3·6 mmol/l. Diuresis was induced by infusion of mannitol and saline (2·5% mannitol in 0·9% NaCl. Infusion rate 7·5 ml/h as prime dose, 4·5 ml/h as continuous infusion). At time zero aldosterone (2 μg/100 g) was infused intravenously. Mean values ± SE. $n = 5$ for each group.

potassium concentration ratio is close to unity. Despite the fact that the net potassium reabsorption in the proximal tubule is under some influence of aldosterone, fractional potassium reabsorption along the proximal tubule of adrenalectomized rats is normal [4, 14, 15].

The main site of action of aldosterone on potassium transport is localized at the distal tubular level. While potassium concentration normally increases along the distal tubule, adrenalectomy reduces this ability [1, 4, 15]. In addition, Cortney [14] has shown that net secretion of potassium along the distal tubule and the collecting duct is reduced in adrenalectomized rats. Thus, it appears that aldosterone is necessary for a normal handling of potassium in the distal tubule. The distal tubule and the collecting duct are mainly responsible for changes in the excretion pattern of potassium.

3. MECHANISM OF ACTION OF ALDOSTERONE ON POTASSIUM TRANSPORT

It has been proposed by Giebisch [16] that the distal transepithelial potential

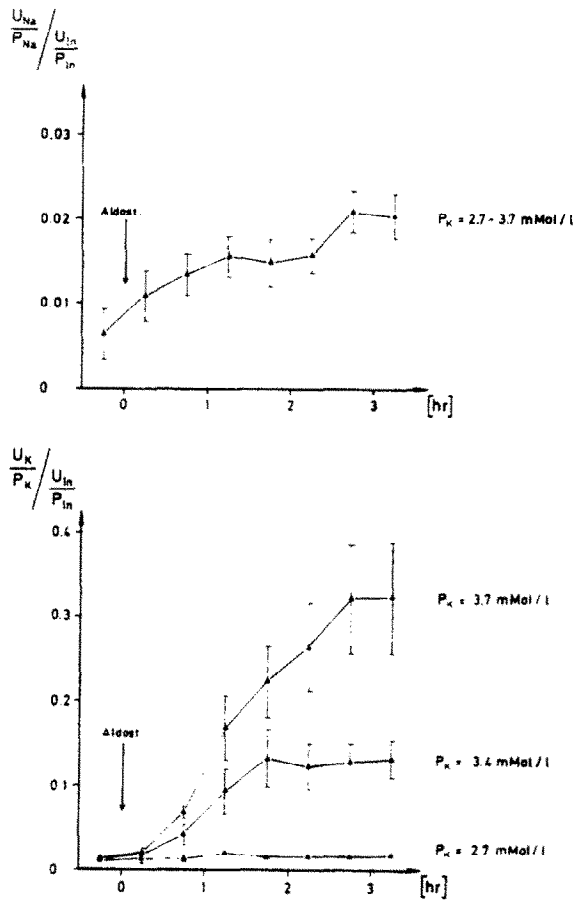

Fig. 2. Effect of aldosterone on sodium and potassium excretion in adrenalectomized rats. Different plasma potassium concentrations were induced by low-potassium diet for various periods (18 h, 24 h, 3 days). Data for sodium excretion were pooled (the difference between the groups was not significant). Mean values ±SE.

difference (P.D.) is the consequence of two potential steps: a diffusion potential across a nearly potassium-selective peritubular membrane and a much smaller potential across a less selective luminal membrane. Estimates of the relative permeability of muscle membrane have been obtained by Hodgkin and Horowicz [17], who observed changes in membrane potential when ionic concentration differences are suddenly altered across the membrane. This method has been adapted to the tubular membranes of the kidney [16, 18, 19].

To investigate the mechanism of action of aldosterone on potassium transport, electrophysiological methods were used in normal and adrenalectomized rats. A schematic representation of the method is shown in Fig. 4: with single or double-barreled pipettes distal tubules were perfused with 150 mmol potassium/l. (anion: sulfate) or 1·5 mmol potassium/l. (anion:sulfate, additional cation:cholin). Measurements of transepithelial P.D. were made in the second half of the distal tubule where the P.D. is constant along the distal segment [19]. The resistance of the electrode was continuously recorded by briefly passing current through the electrode.

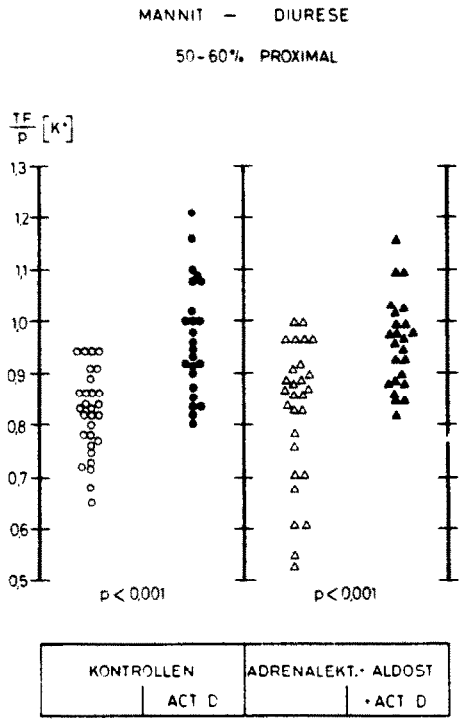

Fig. 3. Free flow micropuncture experiments in osmotic diuresis (5 ml 20% mannitol in 0·9% NaCl/h). Data from 16 rats. Only the last loop of the proximal convolution has been punctured. Determination of potassium concentration (TF = tubular fluid, P = plasma) by microflamephotometry (for methods see [1]). Samples were taken 60–90 min after the onset of the infusion of mannitol. Actinomycin D (200 μg/100 g intravenously) was injected 30 min prior to the mannitol infusion. In adrenalectomized rats aldosterone was given over a period of 3 days (7·5 μg/100 g and 24 h).

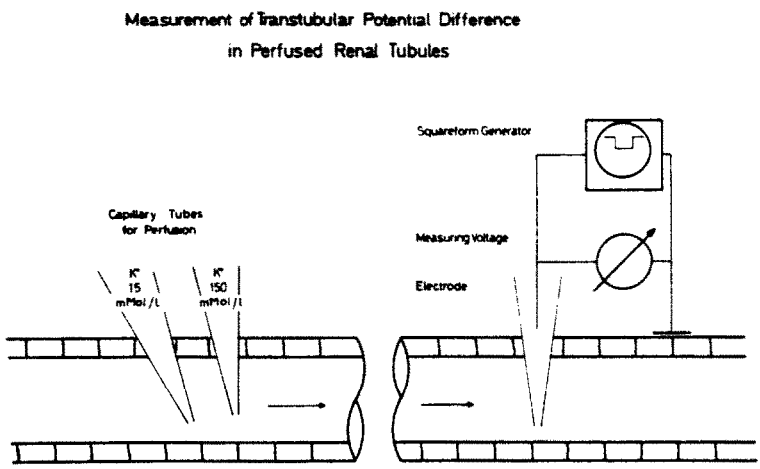

Fig. 4. Schematic presentation of the method of applying various perfusion fluids intratubularly.

In distal tubules of control rats (Fig. 5) the mean transepithelial P.D. was 46·9 mV (lumen negative against peritubular fluid) when the lumen was perfused with 150 mmol potassium/l. This P.D. is almost identical with the P.D. under free flow conditions (non-perfused tubules). When the perfusion fluid was changed to 1·5 mmol potassium/l the P.D. changed within a few seconds to a new steady value of 6 mV (lumen positive). In adrenalectomized rats the transepithelial P.D. was much lower. Again, the P.D. under free flow conditions was in the same range as the P.D. at the luminal perfusion with 150 mmol potassium/l. (mean: 35,1. mV). After a 100-fold change of the luminal potassium concentration the potential change was markedly reduced in adrenalectomized rats compared with control rats. The slope of the potential change was less steep in adrenalectomized rats. In acute experiments administration of aldosterone (2 μg/100 g intravenously) completely normalized the slope of the potential change. The transepithelial P.D. at a luminal perfusion with 150 mmol potassium/l. was almost unchanged by aldosterone.

From the data compiled in Table 1 it can be seen that in control rats the distal transepithelial potential change (Δ P.D.) is 52·9 mV when the luminal potassium concentration is varied by a factor of 100. The potential change in adrenalectomized rats (Δ P.D. 32·5 mV) is significantly reduced as compared with control rats ($P < 0{\cdot}001$). Acute and chronic administration of aldosterone normalized the potential change, both values being significantly different from those in adrenal-

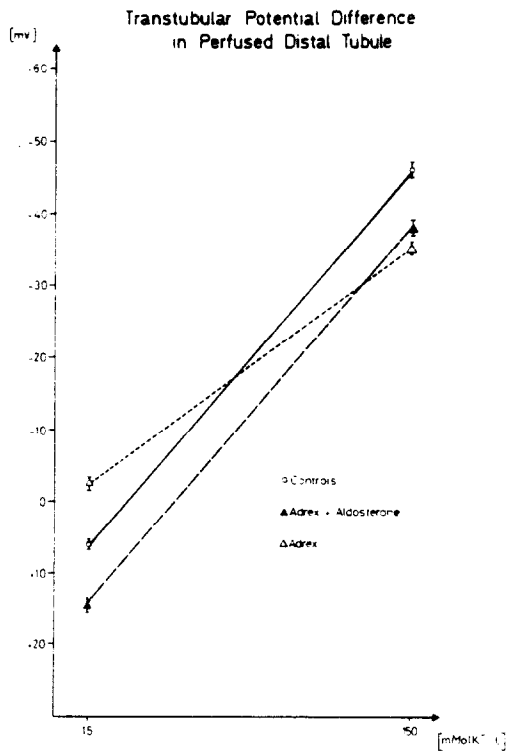

Fig. 5. Effect of luminal microperfusion with 150 and 1·5 mmol potassium/l on transepithelial P.D. in control rats, adrenalectomized rats, and adrenalectomized rats 2–3 h after injection of aldosterone (2 μg/100 g).

Table 1. Effect of luminal [K] on *distal* transepithelial potential difference (mV) and transport number

Rats		Effect of luminal [K] on distal transepithelial P.D. (mV)			
Group	n	150 K	1·5 K	Δ P.D.	Transport number T_K
Controls	9 (111)	−46·9	+6·0	52·9	0·43
Adrex.	7 (102)	−35·1	−2·6	32·5	0·27
Adrex. + aldo. acute	5 (113)	−37·4	+14·2	51·6	0·42
Adrex. + aldo. chron.	6 (86)	−37·0	+9·5	46·5	0·38
Adrex. + aldo. + cyclo.	4 (81)	−44·5	+7·0	51·5	0·42
Controls + cyclo.	3 (68)	−45·5	+4·7	50·2	0·41
Controls + act. D	5 (105)	−39·6	+15·6	55·2	0·45

Values are means. The number of single measurements is given in parentheses.

ectomized rats ($P < 0{\cdot}001$). In another group of experiments cycloheximide (50 μg/100 g intravenously) was given simultaneously with aldosterone. In the experimental period of 2–3 h cycloheximide was unable to prevent the effect of aldosterone on the potential change. Also in control rats, cycloheximide and actinomycin D (100 μg/100 g) did not modify significantly the potential change.

In analysing the potential change when the luminal potassium concentration is varied one has to postulate initially that diffusion potentials contribute to the observed membrane potentials and that the cell ion concentrations are not changed immediately. Assuming that the potentials arise as the sum of diffusion potentials across the luminal and peritubular membranes, transport numbers can be calculated from the various ions[17]. The transport number for potassium (T_K) was calculated as the ratio of the measured change in P.D. per 100-fold change in luminal potassium concentration to the theoretical change in P.D. per 100-fold change in all permeant ions. Furthermore, the transport number indicates the relative permeability (or relative conductance) of the luminal membrane for potassium.

The data compiled in Table 1 demonstrate that the transport number for the distal tubule of adrenalectomized rats is reduced significantly ($P < 0{\cdot}001$) and is normalized by administration of aldosterone. The presented data indicate that the relative permeability of the luminal membrane for potassium is under the influence of mineralocorticosteroids. Inhibitors of protein synthesis in the dose used had no effect on membrane permeability for potassium. From the data presented we postulate that the impairment of distal potassium secretion in adrenal insufficiency is mainly due to a reduced permeability of the luminal membrane. Aldosterone stimulates potassium secretion by increasing the luminal permeability of the distal tubule. The influence of aldosterone on luminal permeability is independent of dietary intake of potassium. Therefore, other additional factors have to be taken in account to explain completely the excretion rates of potassium. Recently it has

been shown[20] that intracellular potassium pool and active uptake of potassium across the peritubular membrane are of importance for the magnitude of potassium secretion.

ACKNOWLEDGEMENTS

This study was supported by the Deutsche Forschungsgemeinschaft. The competent technical assistance of Miss A. Lübbemeier is gratefully acknowledged.

REFERENCES

1. Hierholzer K., Wiederholt M., Holzgreve H., Giebisch G., Klose R. M. and Windhager E. E.: *Pflügers Arch. ges. Physiol.* **285** (1965) 193.
2. Hierholzer K., Wiederholt M. und Stolte H.: *Pflügers Arch. ges. Physiol.* **291** (1966) 43.
3. Wiederholt M., Stolte H., Brecht J. P. und Hierholzer K.: *Pflügers Arch. ges. Physiol.* **292** (1966) 316.
4. Wiederholt M. und Wiederholt B.: *Pflügers Arch. ges. Physiol.* **302** (1968) 57.
5. Wiederholt M. und Hierholzer K.: V. Symposion d. Ges. f. Nephrologie, Lausanne. Springer Verlag, Berlin (1969).
6. Edelman I. S., Bogoroch R. and Porter G. A.: *Proc. Natn. Acad. Sci.* **50** (1963) 1169.
7. Williamson H. E.: *Biochem. Pharmacol.* **12** (1963) 1449.
8. Swingle W. W., Maxwell R., Ben M., Baker C., Lebrie S. J. and Elsler M.: *Endocrinology* **55** (1954) 813.
9. August J. T. and Nelson D. H.: *J. clin. Invest.* **38** (1959) 30.
10. Barger A. C., Berlin R. D. and Tulenko J. F.: *Endocrinology* **62** (1958) 804.
11. Castles T. R. and Williamson H. E.: *Proc. Soc. Expl. Biol. Med.* **119** (1965) 308.
12. Wiederholt M.: *Pflügers Arch. ges. Physiol.* **292** (1966) 334.
13. Fimognari G. M., Fanestil D. D. and Edelman I. S.: *Am. J. Physiol.* **213** (1967) 954.
14. Cortney A.: *Am. J. Physiol.* **216** (1969) 589.
15. Stolte H., Wiederholt M., Fuchs G. and Hierholzer K.: *Pflügers Arch.* **313** (1969) 252.
16. Giebisch G., Malnic G., Klose R. M. and Windhager E. E.: *Am. J. Physiol.* **211** (1966) 560.
17. Hodgkin A. L. and Horowicz P.: *J. Physiol. (Lond.)* **148** (1959) 127.
18. Boulpaep E.: *Transport und Funktion intracellulärer Elektrolyte* (Edited by F. Krück). Urban & Schwarzenberg (1967), Munich, pp. 98–107.
19. Wright F. S.: *Am. J. Physiol.* **220** (1971) 624.
20. Wiederholt M., Sullivan W. J. and Giebisch G.: *J. Gen. Physiol.* **57** (1971) 495.

DISCUSSION

Fanestil: With regard to the data you showed during mannitol diuresis: was the TF/PK$^+$ from micropuncture of proximal tubules?

Wiederholt: Yes, in Fig. 3 micropuncture data are shown: TF/P for potassium at the end of the proximal convolution.

Fanestil: You demonstrated that actinomycin D inhibition in both controls and adrenalectomized animals; have the adrenalectomized animals been treated with aldosterone?

Wiederholt: Yes, adrenalectomized rats have been treated with aldosterone (7·5 μg/100 g B.W.) over a period of 3 days prior to the experiment.

Fanestil: Was there a difference in $\left(\frac{(TF/P)\ Inulin}{(TF/P)\ K^+}\right)$ ratios between adrenalectomized animals and adrenalectomized animals treated with aldosterone?

Wiederholt: I did not show TF/P-potassium data in adrenalectomized rats without hormone substitution. Experiments from our group (Stolte *et al.*, *Pflügers Arch.* **313** (1969) 252) and from Cortney (*Am. J. Physiol.* **216** (1969) 589) demonstrate that in mannitol diuresis TF/P-potassium ratios at the end of the proximal convolution of adrenalectomized rats without hormone substitution are higher than

TF/P-potassium ratios of adrenalectomized rats with aldosterone (Fig. 3). Thus, I would like to postulate that potassium reabsorption in the proximal tubule is to some extent regulated by aldosterone.

Fanestil: There is quite a bit of controversy about the proximal tubular effects of aldosterone and I wonder what your thoughts are about this.

Wiederholt: In adrenal insufficiency net reabsorption of sodium is impaired in the proximal as well as in the distal tubule (Hierholzer *et al.*, *Pflügers Arch.* **291** (1966) 43). This inhibition of sodium transport can be normalized by administration of aldosterone (Wiederholt *et al.*, *Pflügers Arch.* **292** (1966) 316). In contrast to the decreased net reabsorption of sodium fractional reabsorption in the proximal tubule is not reduced in adrenalectomized rats. Various factors (filtered load of sodium, passage time of tubular fluid, tubular geometry . . .) determine the glomerulo-tubular balance of the proximal tubule. Thus, measurement of fractional reabsorption is not a valid method for testing the effect of aldosterone on proximal electrolyte transport.

Snart: Have you tried the effect of glucocorticoids?

Wiederholt: We tried glucocorticosteroids with very low (dexamethasone 50 μg/100 g B.W.) and high (cortisone 2·5 mg/100 g B.W.) mineralocorticoid activity. In the doses used dexamethasone had no effect on sodium transport despite the fact that arterial blood pressure and glomerular filtration rate increased. Net reabsorption of sodium was normalized by cortisone.

Edelman: What was the dose of actinomycin D in these experiments and the time of administration with respect to the micropuncture measurements?

Wiederholt: In experiments on sodium and potassium transport (Fig. 3 and Wiederholt, *Pflügers Arch.* **292** (1966) 334) actinomycin D (200 μg/100 g B.W.) was injected to adrenalectomized rats pretreated with aldosterone for 3 days. In the proximal as well as in the distal tubule the effects of aldosterone on active electrolyte transport were abolished by actinomycin D. In electrophysiological experiments on relative permeability of potassium, actinomycin D (100 μg/100 g B.W.) was administered simultaneously with aldosterone. Aldosterone increased the luminal permeability of the distal tubule for potassium after a time delay of about 2 h. Actinomycin D had no effect on membrane permeability of the distal tubule.

Edelman: What was the time interval between the addition of actinomycin D and the time in which you made your measurements on potassium excretion?

Wiederholt: 2–4 h after the injection of actinomycin D, potassium excretion and membrane permeability were measured.

Edelman: Did you measure the effects of actinomycin D on RNA synthesis?

Wiederholt: No.

Edelman: The studies of Fimognari *et al.* (*Am. J. Physiol.* **213** (1967) 954) indicate that the time-courses of the effect of aldosterone on Na^+ and K^+ excretion were superimposable. At the doses used in this study, actinomycin D inhibited about 65% of RNA synthesis. Thus it is possible that the K^+ effect is also dependent on gene activation but that these gene sites are less sensitive to actinomycin D than those responsible for the Na^+ effect.

Wiederholt: I agree. The dose of actinomycin D we used probably blocks much more than 50% of RNA synthesis. If there are separate gene sites the "potassium excretion regulating gene site" must be less sensitive to inhibitors.