Biochimica et Biophysica Acta, 773 (1984) 246–252
Elsevier

BBA 72118

SOME EFFECTS OF MONOVALENT ANION REPLACEMENT ON THE VOLUME AND COMPOSITION OF CELLS IN INCUBATED SLICES OF RAT RENAL CORTEX

ROBERT O. LAW

Department of Physiology, University of Leicester, Leicester LE1 7RH (U.K.)

(Received December 5th, 1983)

Key words: Cell volume; Water content; Cation; Anion replacement; (Rat kidney)

Steady-state cellular water content and cation content and concentration have been studied in slices of rat renal cortex incubated in media in which Cl^- was replaced by various monovalent anions at pH 7.35. Anions derived from low molecular weight aliphatic acids caused net uptake of cell water and K^+. In the presence of larger anions cell water content fell in a manner related to anionic equivalent weight, but water content in media containing anions from weak aliphatic or alicyclic acids was slightly but consistently higher than that in media containing anions from strong acids. Cell K^+ content did not decrease in these media. Aromatic anions caused enhanced cell water loss. When external pH adjusted to 6.8 or 7.8 it was found that in media containing anions from weak acids, but not from strong acids, increasing pH was associated with significantly decreased cell water content.

Introduction

In studies on mammalian cellular physiology it is sometimes desirable to replace the major extracellular anion, viz. Cl^-, by a supposedly physiologically inert substitute. This may be done, for example, to throw light either upon the role of Cl^- itself on cellular function or the role of an extracellular cation free from the modifying influence of Cl^-. The many examples of this technique in the study of mammalian renal cortical cells include the use of acetate [1], bromide [1,2], cyclamate [3,4], gluconate [5], iodide [1,2], isethionate [1,4,6], methyl sulphate [3,6,7], nitrate [1,2,8] and thiocyanate [2,6,8].

The 'inert' nature of such substituents may be questioned. Some, e.g. acetate, are metabolizable [9]. Others, e.g. salicylate, are transported by renal cortical cells [10]. Any anion whose permeancy is not identical with that of Cl^- may osmotically affect cell water content (i.e. volume). Several anions are derived from weak acids, and low concentrations of free, undissociated acid will therefore be present in the extracellular medium. Cell swelling in the presence of weak acids was recognized nearly a century ago [11], and may be interpreted as cellular penetration by the undissociated species followed by intracellular dissociation into excess osmotically active particles. Anions occupying a place distant from Cl^- in a lyotropic series may influence cell function, e.g. by altering membrane cation permeability [12].

While none of these possibilities will necessarily compromise the course of any specific cellular study, it seems worthwhile to examine and draw attention to certain consequences of anionic substitution. The present report does so in respect of

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Pipes, 1,4-piperazineethanesulphonic acid.

(i) the effects of Cl^- replacement upon steady-state water and cation content at physiological pH (7.35) and (ii) the extent to which the influence of anions on cell volume is dependent upon external pH. Some of these findings have previously been published as an Abstract [13].

Methods

Kidneys from normally hydrated adult male Wistar rats were used. Animals were killed by cervical dislocation and 5–10 slices (thickness approx. 0.3 mm, weight = 5–15 mg) were cut freehand from the renal cortex. Extreme superficial and juxtamedullary tissue was not used. Slices were rinsed briefly in Cl^--Ringer (composition as given below), blotted on hard filter paper (Whatman No. 542), weighed to the nearest 25 μg on a torsion balance and then incubated for up to 150 min in a shaking waterbath. Preliminary experiments showed that no significant alterations in cell water and cation content occurred during a period of 50–150 min; some results from 50-min incubations are accordingly included in Figs. 1 and 2 (see Results).

Slices (in batches not exceeding 5) were incubated in 2.5 ml of medium based on that of Krebs [14], constituted as follows (mmol/l)Na^+ 141, K^+ 6, Ca^{2+} 2.6, Mg^{2+} 1.2, A^- 105, HCO_3^- 25, $H_2PO_4^-$ 2.2, SO_4^{2-} 1.2, pyruvate 4.8, glutamate 4.8, fumarate 5.3, glucose 10, gassed with 95%O_2/5%CO_2 to pH 7.35 at 25°C (where A^- = Cl^-, formate, acetate, NO_3^-, propionate, Br^-, *n*-butyrate, methyl sulphate, isethionate, I^-, salicylate, benzenesulphonate, trichloroacetate, cyclamate, glucuronate or gluconate). Thiocyanate was omitted in view of its known inhibitory action on HCO_3^--stimulated ATPase in rat kidney [15] which, at least in the medulla, impairs cell volume regulation [16].

pH was altered by dropwise addition of 1 M Hepes to approx. 7.8, or solid Pipes to approx. 6.8 (gas phase 100% O_2). All pH measurements were made at 25°C and checked at the end of incubation.

[^{14}C]Carboxyl inulin (Amersham International Ltd.), dissolved in the appropriate medium, was added to the incubates 25 min prior to the end of incubation as an extracellular marker (final activity approx. 20 kBq/ml). Inulin has been widely used as an extracellular marker in renal cortical slices (for review see Ref. 18), and 25 min is sufficient for attainment of equilibrium volume of distribution [17]. Following incubation slices were removed, blotted, weighed and individually leached for 24 h in 2.5 ml deionized water: this procedure is adequate for the complete removal of inulin and ions from small tissue slices [19]. Finally, slices were dried to constant weight at 105°C on tared aluminium foil in order to allow determination of post-incubatory water content and solute-free dry weight.

Inulin activity of leachates, and of a 1:2500 dilution of incubation medium, were measured using a Packard TriCarb Liquid Scintillation Spectrometer, Model 3320. Slice extracellular space (μl) was calculated by simple proportion. Cell water was calculated as (total water − extracellular space) and expressed as μl/mg solute-free dry weight.

Cellular cation concentrations ($[K_i^+]$ and $[Na_i^+]$, mmol/l) were calculated from the formula:

$$[(\text{concn. in total slice water} \times \text{slice water content } (\mu l)) - (\text{extracellular space } (\mu l) \times \text{concn. in incubation medium})] \times (\text{cell water } (\mu l))^{-1}$$

Cellular cation contents (K_i^+ and Na_i^+, nmol/mg solute-free dry weight) were calculated as:

$$[(\text{Cellular concentration (mmol/l)}) \times (\text{cell water } (\mu l/\text{mg solute-free dry weight}))]$$

For Na^+ the apparent concentration in total slice water was appropriately reduced in order to allow for the bound fraction which is present in rat renal cortex (171 nmol/mg solute-free dry weight [20]), and the resultant figure was used in the above calculations.

The terms 'cell water content' and 'cell volume' are considered synonymous in this report (for discussion of this see Ref. 21).

Note also that in the above calculations the specific gravity of renal tissue has been regarded as unity.

Results

In Fig. 1 are shown the steady-state water contents of cortical cells incubated in the presence of various anions. The broken line has no mathematical significance, but indicates the relationship between the volumes attained in the presence of anions derived from strong acids (viz. chloride, nitrate, bromide, iodide and trichloroacetate). The following points may be noted; (1) In the presence of anions from low molecular weight weak acids (formate ($pK_a = 3.77$) *, acetate (4.76), propionate (4.88) and *n*-butyrate (4.82)) cells swell to an extent that their final water contents significantly exceed ($P < 0.001$ for each acid) that attained in Cl^--medium. (2) Increasing anionic equivalent weight is associated with a gradual decrease in cell water content, but values in the presence of aliphatic or alicyclic anions, methyl sulphate, isethionate, cyclamate, glucuronate and gluconate, all lie somewhat above the line relating the anions from strong acids (or its notional extrapolation). For example, the water-content in the presence of isethionate (equivalent weight = 125) is $2.09 \pm 0.07(32)$ μl/mg solute-free dry weight (mean ± S.E. (*n*)) whereas in medium containing iodide (equiv. wt. = 127) the figure is significantly lower ($1.90 \pm 0.04(24)$, $P < 0.05$). (3) Conversely, cells show enhanced water loss, relative to anionic equivalent weight, in the presence of the aromatic anions salicylate and benzenesulphonate. The latter is itself derived from a strong acid ($pK_a = 0.70$), but the cell water content is significantly less than that found in medium containing trichloroacetate ($1.50 \pm 0.05(28)$ vs. $1.66 \pm 0.05(19)$ μl/mg solute-free dry weight, $P < 0.05$) despite identical values of pK_a and closely similar anionic equivalent weights (157 vs. 162).

Cell swelling in the presence of aliphatic weak acids is accompanied by a decrease in the relative volume of extracellular water, as shown in Fig. 2. Conversely there is a decrease in the intracellular/extracellular volume ratio in slices showing cellu-

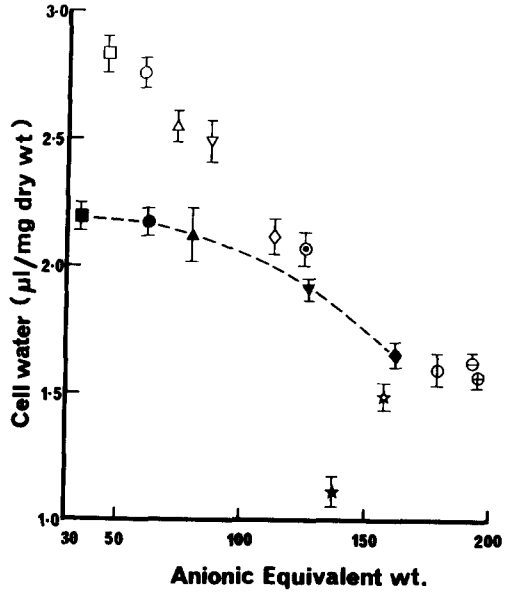

Fig. 1. Steady-state water content of rat renal cortical cells incubated at 25°C and pH 7.35 for 50–150 min in media containing various monovalent anions (105 mmol/l) shown relative to anionic equivalent weight. ■, Cl^-; □, formate; ○, acetate; ●, NO_3^-; △, proprionate ▲, Br^-; ▽, *n*-butyrate; ◇, methyl sulphate; ⊙, isethionate; ▼, I^-; ★, salicylate; ☆, benzenesulphate; ◆, trichloroacetate; Ⓘ, cyclamate; ⊖, glucuronate; ⊕, gluconate. Points are mean ± S.E. ($18 \leqslant n \leqslant 36$).

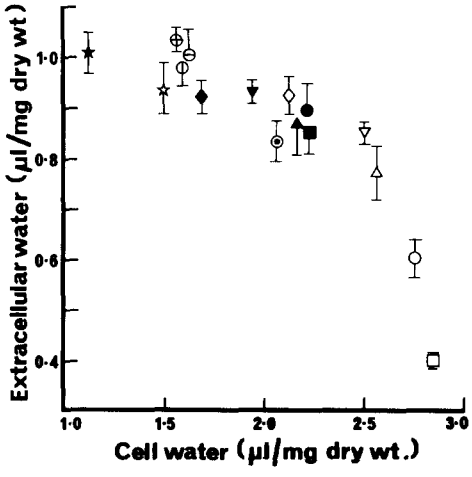

Fig. 2. Steady-state slice extracellular water as a function of cell water (both expressed as μl/mg dry weight) in slices of rat renal cortex incubated at 25°C and pH 7.35 for 50–150 min in media containing various monovalent anions (105 mmol/l). Symbols for each anion are as shown in the legend to Fig. 1. Points are mean ± S.E. (single error bars where these would otherwise conflict) ($18 \leqslant n \leqslant 36$).

* Values for pK_a at 25°C are taken from Organic Chemistry by L.J. Fieser and M. Fieser, 3rd Edn., Reinhold Pub. Co., New York (1956) and from CRC Handbook of Chemistry and Physics (Weast, R.C., ed.), 63rd Edn., CRC Press Inc., Boca Raton, FL (1983).

lar shrinkage. It may be seen from Fig. 2 that whereas in the presence of formate cell water accounts for approx. 87% of total slice water, the intracellular and extracellular volumes are nearly equal in slices incubated in media containing salicylate (cf. that in the presence of Cl^- cell water is approx. 61% of total slice water).

Fig. 3 shows the alterations in K_i^+, $[K_i^+]$, Na_i^+ and $[Na_i^+]$ which accompany the alterations in water content shown in Fig. 1. The most striking feature is the net uptake of K^+ by cells swelling in the presence of formate, acetate, propionate and *n*-butyrate. The mean net gains of K^+ shown in Fig. 3 are 44, 36, 26 and 24 nmol/mg dry wt., respectively. By comparison with cells in Cl^--medium each of these increases is highly significant ($P < 0.001$). Since it is generally regarded that lumens are collapsed in renal cortical slices (see, for example, Ref. 22) this gain must represent K^+ uptake across the peritubular membrane. In media containing other anions (including Cl^- but excluding salicylate) K_i^+ remained constant (within the limits of experimental error) as water content decreased, there being therefore a rise in $[K_i^+]$. Na_i^+ and $[Na_i^+]$ fell steadily as cells lost water, presumably due to Na^+ extrusion across the peritubular membrane. The very marked water loss in the presence of salicylate (Fig. 1) was associated, atypically, with a marked K^+ loss and Na^+ gain.

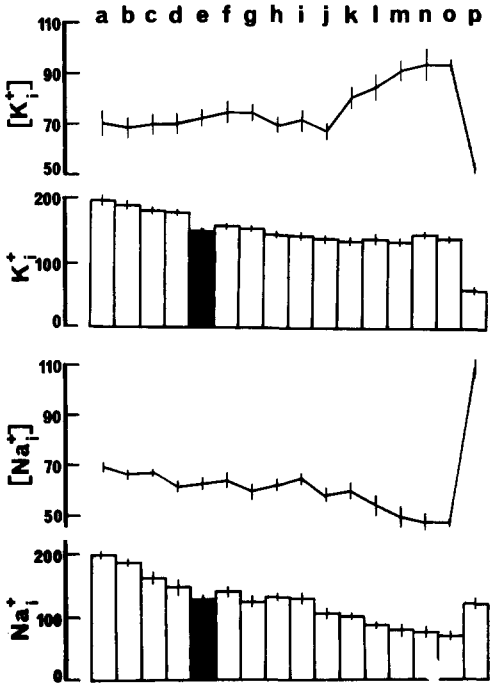

Fig. 3. Steady-state cation concentration ($[K_i^+]$ and $[Na_i^+]$, mmol/l) and contents (K_i^+ and Na_i^+, nmol/mg dry wt.) of rat renal cortical cells incubated at 25°C and pH 7.35 for 50–150 min in media containing various monovalent anions (105 mmol/l). The order in which anions are arranged corresponds to the decreasing cell water contents with which each is associated (Fig. 1), viz. a, formate; b, acetate; c, propionate; d, *n*-butyrate; e (solid bar), Cl^-; f, NO_3^-; g, Br^-; h, methyl sulphate; i, isethionate; j, I^-; k, trichloroacetate; l, glucuronate; m, cyclamate; n, gluconate; o, benzenesulphonate; p, salicylate. Points are mean ± S.E. ($18 \leq n \leq 36$).

It is pertinent to draw attention to the fact that the sum of $[K_i^+]+[Na_i^+]$ in all media fell within the range 130–160 mmol/l. Since the corresponding value in the bathing medium was 147 mmol/l, this range may be regarded as more realistic than the 180–210 mmol/l which is obtained if no allowance is made for the bound fraction of Na^+ in cortical tissue (see Methods).

Effects of altering pH

Table I shows the effects of altering pH to 6.8 or 7.8 upon the steady state water content in the

TABLE I

STEADY-STATE WATER CONTENT OF RAT RENAL CORTICAL CELLS INCUBATED FOR 150 MIN AT pH 6.8 OR 7.8 IN MEDIA CONTAINING ANIONS (105 mmol/l) FROM STRONG AND WEAK ACIDS

Values are mean ± S.E. ($n = 10$). Significance of differences at the two values for pH are represented thus: [a] n.s., * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	Water content (μl/mg solute-free dry weight)	
pH:	6.8	7.8
Strong		
Choride	2.22 ± 0.09	2.17 ± 0.12 [a]
Nitrate	2.07 ± 0.08	2.09 ± 0.10 [a]
Bromide	1.97 ± 0.07	2.07 ± 0.11 [a]
Iodide	2.07 ± 0.07	2.08 ± 0.08 [a]
Trichloroacetate	1.56 ± 0.05	1.68 ± 0.11 [a]
Weak		
Formate	3.13 ± 0.07	2.38 ± 0.08 ***
Acetate	2.88 ± 0.11	2.37 ± 0.11 **
Propionate	2.75 ± 0.10	2.41 ± 0.10 *
n-Butyrate	2.58 ± 0.11	2.08 ± 0.09 **
Gluconate	1.69 ± 0.06	1.15 ± 0.09 ***

presence of five anions derived from strong acids and five from weak acids. Increasing pH causes significant shrinkage in media containing anions from weak acids only.

Discussion

Two main points arise out of the present findings. The first concerns the effects of various anions on cortical cell volume, the significant observation being that in the presence of anions derived from weak aliphatic or alicyclic acids cells accumulate more water than they do in media containing anions derived from strong inorganic or aliphatic acids. This may be manifest either (i) as an absolute increase in water content (i.e. swelling) by comparison with that in 'normal' (i.e. Cl^--containing) medium or (ii) as water content elevated relative to that of cells incubated in the presence of anions of strong acids of comparable molecular weight (Fig. 1). Cell water content in media containing the sodium salts of weak acids (but not strong acids) is pH-dependent (Table I). Secondly, these alterations in cell volume are accompanied by changes in cellular K^+, viz. a net increase in K_i^+ in cells showing absolute increase in water content (without change in $[K_i^+]$) or (independent of the source of the anion, but with the marked exception of salicylate) constancy of K_i^+ and a concommitant rise in $[K_i^+]$ in the presence of anions causing net loss of cell water (Fig. 3). Taken in conjunction, these findings suggest that cell water and K^+ are probably not causally associated; but both, nevertheless, have clear implications in regard to the choice of 'inert' anions as replacements for Cl^-.

Swelling of cells in the presence of weak acids has long been recognized [11]. In medium containing sodium formate, approx. 1 part in 4000 will be present as undissociated formic acid at pH 7.35, i.e. a medium concentration of approx. 26 μmol/l. Higher concentrations of undissociated acids will be present in the case of acids with a higher pK_a (e.g. for propionic acid the corresponding figures are 1 part in 300, approx. 350 μmol/l). On the principle that plasma membranes are very much more permeable to the undissociated than to the ionized species, undissociated acid will diffuse into cells down a chemical concentration gradient. Inside the cells it will dissociate into the ionic species until a steady-state distribution is achieved across the cell membrane. The extent to which osmotically active particles are thus added to intracellular fluids will depend primarily upon (i) the permeability of the cell membrane to the free anion and (ii) the degree of dissociation of the free acid wthin the cell. The latter will depend upon intracellular pH, which in mammalian cortical cells does not normally differ greatly from the external pH (7.2–7.5 [23]). Entry of free acid into the cell, and intracellular dissociation would be expected to lead to a decrease in intracellular pH, although this should be at least partly modified by intracellular buffering.

A decrease in membrane permeability related to increasing anionic equivalent weight, and hence imposition of an increasing external osmotic constraint, may partly explain the relationship shown in Fig. 1. Thus an absolute increase in cell water due to formate, acetate, etc., may represent net addition of osmotically active particles to the cell due to high permeancy (and subsequent dissociation) of the acid. The discrepancy between the water contents in the presence of higher equivalent weight anions from strong and weak acids, all of which cause some degree of cell water loss, results from (i) cellular penetration and dissociation, occurring only in the case of anions of weak acids, and ii) external osmotic constraint due to anions of either type. The excessive water loss due to salicylate and benzenesulphonate may reflect the polar nature of the unsaturated benzene ring (cf. cyclamate) and associated lipid-insolubility of these anions which thus exert an enhanced effective external osmotic pressure. It is notable that the water loss due to benzenesulphonate is greater than that due to trichloroacetate (see Results) despite the steric difference between the two-dimensional benzene ring and the tetrahedral $-CCl_3$ group, a fact which might be expected to afford the former anion a greater permeancy through cell membranes, and hence a lesser effective osmotic affect. Doubtless there are other factors which also influence the permeancy of any given anion, and it may be due to these that, among anions from weak acids, there is no apparent relationship between pK_a and cell water (e.g. formate vs. propionate, vide ultra).

Thus far, the findings confirm and extend the effects of weak acids on cell water, and they indicate that for studies in which maintenance of normal cell volume is desirable, salts of weak acids are unsatisfactory substitutes for Cl^-, particularly if pH changes are involved. Less predictably, they show (Fig. 3) that cell swelling in the presence of such anions can be accompanied by net uptake of K^+. Many previous studies have shown that cortical cell swelling (or whole tissue water uptake) due to a wide variety of causes involves net K^+ loss [24–31]. No explanation for uptake is readily available, and any attempt to interpret the finding is further complicated by the fact that in media containing anions which cause marked shrinkage (except salicylate) cell K^+ is retained at the expense of increased $[K_i^+]$. It must be remembered that the present measurements are of chemical concentration, not intracellular activity, and it is possible that the apparent alterations in K_i^+ and $[K_i^+]$ reflect the effects of anions within the lyotropic series, which can influence intracellular or intramembranous cationic binding (for recent review see Ref. 32). Alternatively, anion-dependent changes in K^+ permeability of the cell membranes may occur directly or indirectly. No parallel fluctuations are apparent in chemically determined levels of Na^+ (Fig. 3) although this would not necessarily be expected since Na^+ binding is mainly extracellular [13]. Detailed further study would be needed to establish the significance, in the present experimental context, of anionic positions within such a series.

The decrease in cell water content at pH 7.8 by comparison with pH 6.8 (Table I) is consistent with the view that in the presence of anions derived from weak acids cellular hydration is partly determined by the availability of undissociated, permeant acid. The higher the pH, the lower the concentrations of indissociated acid in the external medium, there being a 10-fold decrease for a rise of 1 pH unit. Thus at high pH the formation of 'new' intracellular osmotic particles will be diminished and cell swelling reduced. Predictably, no such pH dependence is apparent in the presence of anions from strong acids.

In conclusion, the present findings show that the water content, K^+ content and K^+ concentration within incubated cells of rat renal cortex are influenced by the nature of the major extracellular anion, notably as regards (i) the nature of the acid from which it is derived (weak or strong) and (ii) its equivalent weight, and that these are factors which should be taken into account when medium Cl^- is replaced by another monovalent anion. In terms of maintenance of normal cell water content, nitrate, bromide, methyl sulphate and isethionate appear the most acceptable substitutes; moreover, they have negligible effect on cell K^+ (and Na^+). In an experimental routine involving alterations of external pH only nitrate and bromide are suitable, since methyl sulphate and isethionate are associated with pH-dependent changes in cell water content.

Acknowledgment

I am very grateful to Emeritus Professor R. Whittam, F.R.S., for his helpful comments on this paper.

References

1 Simmons, N.L. (1981) Biochim. Biophys. Acta 646, 231–242
2 Taggart, J.V., Silverman, L. and Trayner, E.M. (1953) Am. J. Physiol. 173, 345–350
3 Neumann, K.H. and Rector, F.C. (1976) J. Clin. Invest. 58, 1110–1118
4 Warnock, D.G. and Yee, V.J. (1982) Am. J. Physiol. 242, F395–F405
5 Whittam, R. (1982) J. Physiol. 324, 54P
6 Dantzler, W.H. and Bentley, S.K. (1981) Am. J. Physiol. 241, F632–F644
7 Jacobson, H.R., Gross, J.B., Kawamura, S., Waters, J.D. and Kokko, J.P. (1976) J. Clin. Invest. 58, 1233–1239
8 Goldinger, J.M., Dew, B., Song, Y.K., Koschier, F.J. and Hong, S.K. (1980) Biochim. Biophys. Acta 598, 357–365
9 Huland, H., Seitz, H.J., Tarnowski, W. and Weiss, C. (1974) Life Sci. 14, 117–128
10 Mudge, G.H., Silva, P. and Stibitz, G.R. (1975) in The Medical Clinics of North America 59, No. 3 (Baruch, S., ed.), pp 681–698, W.B. Saunders, Philadelphia
11 Overton, E. (1902) Pflügers Arch. 92, 115–280
12 Davson, H. (1940) Biochem. J. 34, 917–925
13 Law, R.O. (1983) J. Physiol. 324, 17–18P
14 Krebs, H.A. (1950) Biochim. Biophys. Acta 4, 249–269
15 Kinne-Saffran, E. and Kinne, R. (1974) Proc. Soc. Exp. Biol. Med. (N.Y.) 146, 751–753
16 Law, R.O. (1980) J. Physiol. 307, 475–490
17 Rosenberg, L.E., Downing, S.J. and Segal, S. (1962) Am. J. Physiol. 202, 800–804
18 Law, R.O. (1982) Experientia 38, 411–421
19 George, J.P. and Solomon, S. (1981) J. Physiol. 316, 251–262

20 Law, R.O. (1984) J. Physiol. 346, 101P
21 Macknight, A.D.C. and Leaf, A. (1977) Physiol. Rev. 57, 510–573
22 Giebisch, G., Boulpaep, E.L. and Whittembury, G. (1971) Phil. Trans. R. Soc. Lond. B. 262, 175–196
23 Boron, W.D. and Sackin, H. (1983) Annu. Rev. Physiol. 45, 483–496
24 Whittam, R. and Davies, R.E. (1953) Biochem. J. 55, 880–888
25 Cort, J.H. and Kleinzeller, A. (1956) J. Physiol. 133, 287–300
26 Robinson, J.R. (1965) J. Physiol. 177, 112–121
27 Macknight, A.D.C. (1968) Biochim. Biophys. Acta 163, 85–92
28 McIver, D.J.L. and Raine, A.E.G. (1972) J. Physiol. 225, 555–564
29 Hughes, P.M. and Macknight, A.D.C. (1977) J. Physiol. 267, 113–136
30 Cooke, K.R. (1978) J. Physiol. 279, 361–374
31 Pine, M.B., Rhodes, D., Thorp, K. and Tsai, Y. (1979) J. Physiol. 297, 387–403
32 Dani, J.A., Sanchez, J.A. and Hille, B. (1983) J. Gen. Physiol. 81, 255–281