*Biochimica et Biophysica Acta*, 382 (1975) 204–212


BBA 76883

# NET $Cl^-$ FLUX IN SHORT-CIRCUITED SKIN OF *RANA PIPIENS*: OUABAIN SENSITIVITY AND $Na^+ + K^+$ DEPENDENCE

C. O. WATLINGTON and F. JESSEE, Jr

*Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Va. 23298 (U.S.A.)*

(Received July 19th, 1974)
(Revised manuscript received November 11th, 1974)

## SUMMARY

Skin of *Rana pipiens*, like many other species, is considered to actively transport only $Na^+$ when bathed in Ringer's solution on both sides. However, net $Cl^-$ influx was previously described by us in short-circuited skin of *Rana pipiens*, in the summer season. The skins were of low PD (5–30 mV) and high $Cl^-$ conductance. Comparison of these findings with other series indicated inverse seasonal variation between PD and $Cl^-$ conductance. It was postulated that active $Cl^-$ transport exists at all $Cl^-$-conductance levels but at higher PD is too small to be be easily detected. This report evaluates $Cl^-$ transport across skins of higher PD (and lower conductance) in winter and further characterizes the system. Net $Cl^-$ influx was demonstrated over a wide PD range under short-circuit conditions and was inversely related to the magnitude of the open-circuit PD. It was inhibited by ouabain and dependent upon $Na^+$ in the outside medium and $K^+$ in the inside medium. It is concluded that this is the same system present in skin of *Leptodactylus ocellatus* and may not be unique to these two species. It appears to be distinctly different from the acetazolamide-sensitive influx system seen in "low-$Cl^-$" solutions in skins of numerous species. The findings are discussed in terms of (1) parallel $Na^+$ and $Cl^-$ active transport mechanisms and (2) a neutral NaCl-pump model, recently proposed by Rehm.

## INTRODUCTION

Ussing and coworkers [1–3] and Linderholm [4] first presented evidence in isolated skin of *Rana temporaria* and *Rana esculenta* that $Na^+$ is actively transported and $Cl^-$ transported passively. The following findings were presented as proof of passive transport of $Cl^-$: (1) The flux ratio of $Cl^-$ approximated that expected for a given electrochemical gradient across the skin; (2) The short-circuit current, i.e. the electrical current across the skin in the absence of an electrical gradient and with

Abbreviation: s.c.c., short-circuit current.

equal concentrations of ions on both sides of the skin, was equivalent to the net $Na^+$ flux or $Na^+$ current. The assumption has generally been made, for the species *Rana pipiens*, that $Cl^-$ transport is also passive since equivalence between short-circuit current (s.c.c.) and net $Na^+$ flux has been repeatedly demonstrated in isolated skin.

However, experiments in this laboratory, in living anesthetized *R. pipiens* with a wide range of spontaneous open-circuit potential difference (PD), demonstrated that the short-circuit current was clearly less than net $Na^+$ flux in skins with PD values below 20–30 mV [5]. Active inward $Cl^-$ transport across the skins of low PD was postulated to explain this discrepancy. In an extension of these studies in a series of unselected isolated skins of *R. pipiens* in summer, with a mean potential difference (PD) of 13 mV (5–30 mV), the short-circuit current equivalent was found to be significantly less than net $Na^+$ flux [6]. No discrepancy was found in absence of $Cl^-$ (skins bathed in sulfate Ringers'). Net $Cl^-$ influx was demonstrated in $Cl^-$-containing Ringers' solution and was of appropriate magnitude to explain the discrepancy.

Comparison of these findings with small series of unidirectional flux measurements performed at other seasons suggested (1) that passive $Cl^-$ conductance is high in summer, decreases in fall and winter and increases again in spring and (2) that an inverse seasonal relationship between PD and $Cl^-$ conductance also existed. It was postulated that active $Cl^-$ transport exists at all $Cl^-$ conductance levels but at higher PD values is too small to be easily detected by the short-circuit current technique.

This study evaluates $Cl^-$ transport across frog skin at higher PD's in the winter season and further characterizes the system. Net $Cl^-$ influx was demonstrated over a wide PD range under short-circuit conditions and was inversely related to the magnitude of the open circuit PD. It was inhibited by ouabain and dependent upon $Na^+$ in the outside medium and $K^+$ in the inside medium.

## METHODS

Unfed 40–60 g male and female frogs (*R. pipiens*) that were kept in large bins with running water were used. Abdominal skin pairs were dissected from decapitated frogs and mounted between Lucite hemichambers. The volume of the compartments on each side of the skin was 8 ml and the skin area was 1.33 $cm^2$. The experimental system is described in more detail elsewhere [6]. The electrolyte solution bathing the skin, in most experiments, had the following composition in mmol/l: NaCl, 113; KCl, 3.0; $NaH_2PO_4$, 2.0. The solution was adjusted to pH 7.40. $Na^+$-free solution contained arginine chloride 115, KCl 1.0 and $KH_2PO_4$ 2.0 mM. $K^+$-free solution was the same as the $Na^+$-containing solution except for replacement of KCl by 3 mM arginine chloride.

The skin was continuously short-circuited except for open-circuit state 30 s of every 6 min. The PD and s.c.c. in Tables I and II are the values taken at the midpoint of the 1-h flux period in question. The d.c. conductance ($G$) was estimated from the equation $G$ = s.c.c./PD. Marked quantitative variation in $Cl^-$ flux from skin to skin presents a problem in attempts to demonstrate net $Cl^-$ influx [6]. Even unidirectional $Cl^-$ flux in the same direction in skin pairs can be quite different in magnitude [7]. This difference may be due to variable skin tension or edge damage

in mounting and/or mosaic behavior of the skin. However, $Cl^-$ conductance or flux appears to roughly correlate with PD in an inverse manner [1, 6, 8]. Therefore, PD was used as a criterion to improve matching. Skin pairs were not used if the lower PD of the pair was less than 50 % of the higher measurement. This rather lenient range of PD within pairs was arbitrarily allowed because PD is not just a function of $Cl^-$ conductance [8]. It is, at least, a function of active $Na^+$ transport as well.

At the beginning of each experiment, $^{36}Cl^-$ was added to the inside or outside solutions of one of each pair to perform simultaneous influx and outflux determinations. Flux measurements were begun 1 h after mounting. $^{36}Cl^-$ was counted in a liquid scintillation spectrometer using a liquid scintillator in naphthalene-dioxane.

## RESULTS

Table IA presents the results of $Cl^-$ flux and electrical measurements on two unselected series of skin pairs performed in January and March, respectively. In each case a significant net $Cl^-$ influx was demonstrated under short-circuit conditions. Grouping of all experiments from the two series according to PD range (Table IB) reveals net $Cl^-$ influx at low and high PD values. The net influx was significant in the series below 30 mV (mean of 19 mV) and above 49 mV (mean of 64 mV). Mean influx, outflux, net flux and $G$ were all less in the higher PD range. This inverse relationship of $Cl^-$ flux to PD is even more evident when the values in this study are compared to control values in Table IIB and with those of the previous series [6] which had a mean PD of 13 mV. In the latter series the influx, outflux and net flux were 4.71, 3.75 and 0.96 $\mu$equiv $\cdot$ $cm^{-2} \cdot h^{-1}$, respectively. Also, the average s.c.c. within the two series was greater at higher average PD values, as previously noted for the single series of measurements in vivo [5]. Correction of the s.c.c. for the model $Cl^-$ current demonstrated does not account for this latter correlation so that the $Na^+$ transport must indeed be higher at higher PD, within a series.

The response of the net $Cl^-$ transport system to ouabain and $Na^+$- and $K^+$-free solutions is shown in Table II. Ouabain (Table IIA), 0.1 mM, was placed on the skin inside at the end of the 1st hour flux period in 13 of the skins pairs studies in March 1974 (Table IA). A subsequent 1-h flux period was performed. The net $Cl^-$ flux was reduced to insignificant levels. This was accomplished by an increase in $Cl^-$ outflux (See Discussion).

The series assessing electrolyte dependence of $Cl^-$ flux were performed in May and June 1974 (Table IIB). The control influx, outflux and net flux are all substantially greater than these values in the series in Table I. This seasonal variation is expected [6]. With $Na^+$-free solution on the skin outside, net $Cl^-$ flux was minimal compared to the control series. Both influx and outflux were significantly less (46 and 40 %, $P < 0.005$ and $< 0.01$, respectively). Similarly, with $K^+$-free medium bathing the skin inside, net $Cl^-$ flux was not significantly different from zero. However, only $Cl^-$ influx was significantly lower than in the control series ($P$ 0.05). Short-circuit current was significantly lower than control, both in the presence of $Na^+$-free solution on the outside and $K^+$-free solution on the inside. ($P$ 0.001 and 0.005, respectively).

TABLE I

$Cl^-$ FLUX AND ELECTRICAL MEASUREMENTS IN ISOLATED SHORT-CIRCUITED FROG SKIN PAIRS

$Cl^-$ influx and outflux were performed on skin pairs and net flux calculated for each pair. *P* refers to Student's *t* test of net flux treating in and outflux as paired data. *G* refers to d.c. conductance. All values represent mean ±S.E. Electrical measurements were averaged for each skin pair and then mean values calculated from the pair average for each series (See Methods).

| | *N* | Influx | Outflux | Net flux | *P* | s.c.c. | PD | *G* |
|---|---|---|---|---|---|---|---|---|
| | | | ($\mu$equiv · $cm^{-2}$ · $h^{-1}$) | | | ($\mu A \cdot cm^{-2}$) | (mV) | ($k\Omega^{-1} \cdot cm^{-2}$) |
| A. According to experimental data | | | | | | | | |
| Jan. '74 | 36 | 0.705±0.071 | 0.466±0.044 | 0.239±0.066 | <0.001 | 31.0±2.68 | 37.4±2.79 | 0.88±0.049 |
| Mar. '74 | 22 | 0.783±0.15 | 0.525±0.093 | 0.261±0.076 | <0.005 | 44.5±4.09 | 48.1±4.79 | 1.08±0.097 |
| B. According to PD range | | | | | | | | |
| <30 mV | 18 | 1.050±0.170 | 0.650±0.099 | 0.400±0.088 | <0.001 | 20.1±1.70 | 19.4±1.50 | 1.13±0.099 |
| 30–49 mV | 21 | 0.693±0.102 | 0.510±0.070 | 0.183±0.099 | <0.10 | 35.6±3.66 | 39.6±1.25 | 0.99±0.084 |
| >49 mV | 19 | 0.482±0.060 | 0.309±0.031 | 0.173±0.016 | <0.001 | 52.1±3.24 | 64.4±2.66 | 0.82±0.048 |

TABLE II

EFFECT OF OUABAIN AND $Na^+$ AND $K^+$ REPLACEMENT ON $Cl^-$ FLUX IN SHORT-CIRCUITED SKIN PAIRS

$Cl^-$ influx and outflux were performed on skin pairs and net flux calculated for each pair. *P* refers to Student's *t* test of net flux treating in and outflux as paired data. All values represent mean ± S.E. In Series A, electrical measurements were averaged for each skin pair and then mean values calculated from the pair average for each series (See Methods). In Series B mean ± S.E. was not calculated from the pair averages but for the total number of skins studied e.g. for the control series, $N = 50$ for PD and s.c.c.

| Conditions | *N* | Influx | Outflux ($\mu$equiv · $cm^{-2}$ · $h^{-1}$) | Net flux | *P* | s.c.c. ($\mu A \cdot cm^{-2}$) | PD (mV) |
|---|---|---|---|---|---|---|---|
| A. Effects of 0.1 mM ouabain | | | | | | | |
| Before ouabain | 13 | 0.804 ± 0.252 | 0.507 ± 0.142 | 0.297 ± 0.119 | < 0.05 | 40.3 ± 5.36 | 49.2 ± 7.33 |
| After ouabain | 13 | 0.864 ± 0.194 | 0.823 ± 0.158 | 0.041 ± 0.081 | N.S. | 16.5 ± 2.02 | 16.5 ± 2.02 |
| B. $Na^+$ and $K^+$ replacement | | | | | | | |
| Control | 25 | 2.50 ± 0.347 | 1.92 ± 0.214 | 0.58 ± 0.225 | < 0.02 | 29.9 ± 2.07 | 18.2 ± 1.86 |
| $Na^+$-free solution outside | 22 | 1.34 ± 0.167 | 1.16 ± 0.176 | 0.18 ± 0.176 | N.S. | 14.2 ± 1.06 | 12.9 ± 1.15 |
| $K^+$-free solution inside | 19 | 1.66 ± 0.246 | 1.69 ± 0.251 | 0.03 ± 0.253 | N.S. | 21.0 ± 1.97 | 15.2 ± 1.40 |

## DISCUSSION

The experiments here reported indicate net $Cl^-$ flux inward under short-circuit conditions in isolated skin of *R. pipiens* in winter season and at high PD and low $Cl^-$ conductance or permeability. As originally speculated, from studies in summer on skin of low PD and relatively high $Cl^-$ conductance [6], the net flux is on average inversely related to the PD and of small enough magnitude to be difficult to detect. For instance, in the two series performed in January and March (Table I) the average $Cl^-$ current was approximately 4 $\mu A \cdot cm^{-2}$ compared to average s.c.c. at midpoint of the flux periods of 46 and 31 $\mu A \cdot cm^{-2}$, respectively. This approximate 10 % contribution of net $Cl^-$ flux to s.c.c. could go unobserved if $Cl^-$ flux determinations of small number were made or if inference of absence of net $Cl^-$ flux was based on equivalence between s.c.c. and net $Na^+$ flux. This small contribution would more easily be over-looked should there be selection of skins of relatively high PD for experimental purposes. For instance, the average $Cl^-$ current as percent of s.c.c. in the groupings above 29 mV were only 8 and 5 %, for the ranges 30–49 mV and $>$ 49 mV, respectively. Thus, approximate equivalence between s.c.c. and net $Na^+$ flux would be present.

In all flux measurements the skins were under open-circuit conditions for 30 s of every 6 min. Thus, 8 % of the time a potential gradient favorable for net $Cl^-$ influx was present. Conceivably, this gradient could account for part or all of the net $Cl^-$ influx since the flux ratios for $Cl^-$ are relatively small. This possibility was tested using the Goldman-Hodgkin-Katz equation as applied to unidirectional fluxes [9]. The expected flux ratio ($J_i/J_0$), assuming a completely passive behavior for $Cl^-$ (influx = outflux) 92 % of the time and an electrical field across the skin 8 % of the time, was calculated for all the mean PD values in Table I. In every case the calculated ratio was substantially less than the ratio found. For instance, for PD values of 19.4 and 64.4 mV (Table IB) the calculated $J_i/J_0$ values were 1.06 and 1.22, respectively. The experimental $J_i/J_0$ values were 1.62 and 1.56, respectively. Thus, the brief periods of open-circuit state do not account for most of the net $Cl^-$ influx, within the limitations of the assumptions inherent in the Goldman-Hodgkin-Katz equation. We conclude that active $Cl^-$ transport inward does exist.

The inhibition of net $Cl^-$ influx produced by ouabain (Table IIA) deserves some discussion. Outflux of $Cl^-$ increased, but little change in influx occurred. The increase in $Cl^-$ outflux is similar to effects of ouabain on $Na^+$ outflux in frog skin [10]. We conclude that active $Cl^-$ influx was indeed depressed while passive $Cl^-$ influx increased, thus producing little over-all change in influx. However, it could be argued that ouabain did not alter active $Cl^-$ transport inward, but affected outward movement of $Cl^-$ alone. Against the latter argument is our subsequent finding that ouabain often decreases $Cl^-$ influx in skins with higher influx values (to be published elsewhere). This suggests that in the presence of a larger component of active $Cl^-$ influx the inhibitory effect of ouabain is no longer completely masked by an increase in passive flux.

In skins bathed in $K^+$-free solution on the inside (Table IIB), net $Cl^-$ flux was not detectable. This effect was the result of a decrease in $Cl^-$ influx. Thus, net $Cl^-$ influx resembles active $Na^+$ transport not only in being ouabain sensitive but also in dependence upon $K^+$ [11, 12]. A similar or identical ATPase could be responsible

for transport of the two ions. (See below).

In the presence of $Na^+$-free solution on the skin outside, net $Cl^-$ influx was markedly reduced (Table IIB). However, in contrast to results in $K^+$-free solution, both $Cl^-$ influx and outflux were significantly less than in control skins. Macey and Myers [13] found a 45 % decrease in $Cl^-$ influx in isolated skin of *R. pipiens* upon complete replacement of $Na^+$ with $K^+$ in the outside bathing solution compared to a 46 % difference in this study. They did not determine $Cl^-$ outflux. Both $Cl^-$ influx and outflux were approximately 80 % less in isolated short-circuited skin of *Leptodactyllus ocellatus* [14], bathed on both sides by $Na^+$-free choline-containing Ringers' solution, compared to skins in $Na^+$-containing Ringers' solution. Dependence of $Cl^-$ influx and net flux on presence of $Na^+$ in the outer medium could be explained by coupling of the two ions in entry to transporting cells (as postulated for small intestine [15]) or coupled active transport [16] (see below). However, neither mechanism explains dependence of $Cl^-$ outflux upon presence of $Na^+$ outside. Possibly, removal of outside $Na^+$ alters characteristics of a major barrier(s) to $Cl^-$ movement in either direction.

Upon replacement with a $K^+$-free solution inside (Table IIB) a substantial residual s.c.c. of 21 $\mu A \cdot cm^{-2}$ was present. Certainly, there is some $K^+$ available for local recycling to support a residua of active $Na^+$ transport. This $K^+$ would originate from leakage out of epidermal cells. The residual s.c.c. in the presence of $Na^+$-free arginine chloride solution outside is more difficult to explain. Positive current inward could be the result of an arginine diffusion current, active arginine transport or a combination of these. Pinschmidt, et al. [17] found essentially no s.c.c. in skins of *R. pipiens* bathed in arginine chloride solution on the outside. Their experiments were performed between October and March when electrical conductance is relatively low. It may be that substantial active or passive flux of arginine chloride occurs only in spring and summer i.e. periods of high conductance.

This net $Cl^-$-transport system appears similar to that reported in the species *L. ocellatus* as the two share the property of ouabain sensitivity [18] and dependence on $Na^+$ [14]. Also the magnitude of unidirectional $Cl^-$ flux and net flux in our original demonstration of net $Cl^-$ flux in summer [6] was similar to the finding in *L. ocellatus* [18] as was the PD range (5–30 and 10–40 mV, respectively). Thus, net $Cl^-$ flux in "high"-$Cl^-$ Ringer's solution is not unique to the South American frog, but may be present in frogs of all or many species but most easily demonstrable only when $Cl^-$ permeability is relatively high. It appears distinct from the acetazolamide sensitive net $Cl^-$ transport inward which is demonstrable in skin bathed in low $Cl^-$ solution (1–3 mM) in several species, including *R. pipiens* [19]. Kristensen [20] has demonstrated absence of ouabain sensitivity and independence of presence of $Na^+$ in the bathing medium in this latter system in skins of *R. temporaria*. In contrast, we have found no effect of high concentration of acetazolamide on the ouabain sensitive system reported here (studies to be published elsewhere).

One possible explanation of this apparent active $Cl^-$ transport which is ouabain sensitive and dependant upon $Na^+$ in the outside medium and $K^+$ in the inside medium is the coexistence of parallel $Na^+$ and $Cl^-$ pumps, each coupled to metabolism by a ($Na^+ + K^+$)-dependant, ouabain-sensitive ATPase. The magnitude of active $Cl^-$ transport would be expected to be dependent on $Cl^-$ entry from the outer medium. Thus, under conditions of high $Cl^-$ permeability the contribution of

$Cl^-$ current to net ion transport would be significant. The net active $Cl^-$ transport would be lower at lower permeability or conductance, the latter conditions existent at higher PD [1, 8].

Another possible explanation of this phenomenon has been suggested by Rehm et al. [16] utilizing a model containing a neutral NaCl pump. This double membrane model has parallel $Na^+$- and $Cl^-$-conductive pathways, only, in the outside membrane and parallel $K^+$ and $Cl^-$ conductive pathways, only, in the inner membrane. These assumptions are qualitatively in keeping with findings in frog skin [21, 22]. The neutral NaCl pump is placed on the inner membrane with active transport directed inward. When NaCl transport occurs under open-circuit conditions at steady state, all $Cl^-$ actively moved through the neutral NaCl pump must originate in the outside solution. Under short-circuit conditions the $Na^+$ for active transport must still enter from the outside solution as the inner membrane is relatively impermeable to this ion. However, the neutral pump may now receive $Cl^-$ from both outside and inside bathing solutions. The latter $Cl^-$ is non-isotopic in influx determination and simply recycles from medium to cell interior to pump and back to inside solution, and is not noted in transepithelial flux measurements. The fraction of $Cl^-$ passing through the pump from outside and inside respectively becomes a function of the relative conductance for $Cl^-$ of the two membranes. Thus, when the conductance of the outer membrane is much less than the conductance of the inner membrane, net $Cl^-$ current is present but insignificant compared to the $Na^+$ current and most of the $Cl^-$ for the pump arises from the inner bathing medium. In other words, under the artifice of short-circuit conditions, little transepithelial net $Cl^-$ flux is present despite the fact that neutral NaCl is being actively transported. This would be analogous to the condition of skins of high PD and low $Cl^-$ conductance (Table I). At higher relative $Cl^-$ conductance of outer to inner membrane more of the pump $Cl^-$ originates in the outer medium and the net $Cl^-$ flux or current increases to significantly contribute to the s.c.c., as found in summer frogs of high $Cl^-$ conductance and low PD [6].

Since the model predicts much or most of the pump $Cl^-$ arising from the inner solution, under short-circuit conditions, dependence of active $Na^+$ transport upon the nature of the anion present on the skin inside is expected, unless the outer membrane $Cl^-$ permeability is quite high relative to inside. Huf [23] has demonstrated a marked reduction in s.c.c., $Na^+$ influx and net $Na^+$ flux when $Cl^-$ inside is replaced with $SO_4^{2-}$, an anion of very low permeability. The s.c.c. is partially reduced when $Cl^-$ inside is replaced by other less permeant monovalent anions, $HCO_3^-$ or $NO_3^-$. Indeed, recycling of $Cl^-$ coupled with active $Na^+$ transport has been suggested by Pinschmidt et al. [17] as a possible explanation of the $Cl^-$ dependence of catecholamine stimulated active $Na^+$ transport [17].

The neutral NaCl pump model of Rehm et al. is appealing because it accounts for the following, utilizing one active transport mechanism: (a) active $Na^+$ and $Cl^-$ transport which is $Na^+ + K^+$ dependant and ouabain sensitive; (b) variation of net $Cl^-$ flux with $Cl^-$ conductance, under short-circuit conditions; and (c) dependence of $Na^+$ transport inward upon the nature of anions in the inner solution, under short-circuit conditions. Neutral active NaCl transport does not necessarily deny $K^+$ participation in the energy transduction step with ATP and is thus compatible with this requirement of active transport. A neutral active NaCl transport certainly cannot

account for all $Na^+$ transport. Residual active $Na^+$ transport has repeatedly been shown in skins bathed in sulfate Ringers' solution on both sides (cf refs 6 and 23).

ACKNOWLEDGEMENT

This investigation was supported by National Institute of Health Research Grant 1-RO1-AM 16564.

REFERENCES

1 Koefoed-Johnson, V., Levi, H. and Ussing, H. H. (1952) Acta Physiol. Scand. 25, 150–163
2 Koefoed-Johnson, V., Ussing, H. H. and Zerahn, K. (1952) Acta Physiol. Scand. 27, 38–48
3 Ussing, H. H. and Zerahn, K. (1951) Acta Physiol. Scand. 23, 110–127
4 Linderholm, H. (1952) Acta Physiol. Scand. 27, Suppl. 97, 1–144
5 Watlington, C. O., Burke, P. K., Campbell, A. D. and Huf, E. G. (1964) J. Cell Comp. Physiol. 64, 389–408
6 Watlington, C. O. and Jessee, F., Jr (1973) Biochim. Biophys. Acta 330, 102–107
7 Watlington, C. O. and Taylor, L. B. (1974) Comp. Biochem. Physiol., in the press
8 Linderholm, H. (1953) Acta Physiol. Scand. 28, 211–217
9 Mandel, L. J. and Curran, P. F. (1972) J. Gen. Physiol. 59, 503–518
10 Biber, T. U. L., Chez, R. A. and Curran, P. F. (1966) J. Gen. Physiol. 49, 1161–1176
11 Huf, E. G. and Wills, J. (1951) Am. J. Physiol. 167, 255–260
12 Ussing, H. H. (1954) in Ion Transport Across Biological Membranes, (Clarke, H. P., ed.), pp. 3–22, Academic Press, New York
13 Macey, R. I. and Meyers, S. (1963) Am. J. Physiol. 204, 1095–1099
14 Zadunaisky, J. A. and DeFish, F. W. (1964) Am. J. Physiol. 207, 1010–1014
15 Nellans, H. N., Frizzell, R. A. and Schultz, S. G. (1973) Am. J. Physiol. 225, 467–475
16 Rehm, W. S., Hoffman, F. M. and Sanders, S. S. (1974) The Physiologist 17, 320
17 Pinschmidt, M. W., Campbell, A. D. and Huf, E. G. (1973) Biochim. Biophys. Acta 323, 309–325
18 Zadunaisky, J. A., Candia, O. A. and Chiarandini, D. J. (1963) J. Gen. Physiol. 47, 393–402
19 Martin, D. W. and Curran, P. F. (1966) J. Cell Physiol. 67, 367–374
20 Kristensen, P. (1972) Acta Physiol. Scand. 84, 338
21 Fukuda, T. R. (1944) Jap. J. Med. Sci., Part III, Biophysics 10, 77–86
22 Koefoed-Johnson, V. and Ussing, H. H. (1958) Acta Physiol. Scand. 42, 298–308
23 Huf, E. G. (1972) Acta Physiol. Scand. 84, 366–381