Biochimica et Biophysica Acta 821 (1985) 72–78
Elsevier

BBA 72829

Temperature sensitivity of potassium flux into red blood cells in the familial pseudohyperkalaemia syndrome

Michael Meenaghan [a], G. Frederick Follett [b] and Peter J. Brophy [a,*]

[a] *Department of Biological Science, University of Stirling, Stirling FK9 4LA and* [b] *Department of Clinical Chemistry, Falkirk and District Royal Infirmary, Falkirk FK1 5QE (U.K.)*

(Received May 29th, 1985)
(Revised manuscript received August 5th, 1985)

Key words: Familial pseudohyperkalemia; K^+ flux; Temperature sensitivity; Phospholipid; (Human erythrocyte)

The temperature dependence of potassium flux into the red cells of normal and pseudohyperkalaemic individuals over the range 4–40°C was measured using $^{86}RbCl$ as tracer. Flux through the pump was measured as the ouabain-sensitive component (0.2 mM ouabain) and flux via Na^+,K^+-cotransport was measured as the decrease in the rate of K^+ influx in the presence of 1 mM furosemide. The residual passive permeability of the red cell plasma membranes to K^+ was that influx which was unaffected by either inhibitor. When Na^+ influxes were measured, the ratio of Na^+ to K^+ transported via the furosemide-sensitive component was 1 over the full temperature range studied. The temperature sensitivity of K^+ influx via the pump was normal as was the enzymic activity of the Na^+,K^+-ATPase. In contrast, the activity of the Na^+,K^+-cotransport system in pseudohyperkalaemics was more temperature sensitive than that of controls and affected individuals also showed a greater passive permeability to K^+ at low temperatures. Red cell membranes from affected individuals have significantly increased amounts of phosphatidylcholine which are balanced, to a degree, by a decreased content of phosphatidylethanolamiane. It is proposed that in this example of familial pseudohyperkalaemia there is an alteration in the structure of the red cell plasma membrane which influences the temperature sensitivity of both its cotransport and passive permeability properties.

Introduction

There are three major components of K^+ flux in human red blood cells: the Na^+,K^+-ATPase pump, the Na^+,K^+-cotransport system, and the passive diffusion of K^+ across the membrane due to the residual permeability of the red cell plasma membrane. These three components can be distinguished by the use of appropriate inhibitors: ouabain for the pump [1] and loop diuretics such as furosemide for cotransport [2]. The residual passive leak is defined as the flux which is insensitive to both inhibitors. Abnormalities in one or more of these components of K^+ flux have been shown to be present in a variety of cnditions, including Rh_0 erythrocytes [3], hereditary stomatocytosis syndrome [4] and β-thalassaemia minor [5].

The familial pseudohyperkalaemia syndrome was first described by Stewart et al. [6]. The red cells of affected individuals are abnormally leaky to K^+ when kept at low temperatures and the magnitude of the leak increases as the temperature is lowered, particularly below 20°C. The incidence

* To whom correspondence should be addressed.

Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

of the condition in the first family studied was consistent with an autosomal dominant mode of inheritance. The syndrome has since been identified in a family in France [7] and in one in South Wales [8]. We report here our studies on a family from the Stirling area, some of whose membranes show the cold-induced loss of K^+ that is characteristic of the familial pseudohyperkalaemic syndrome.

Materials and Methods

Preparation of ghosts for Na^+,K^+-ATPase assay. Fresh venous blood was collected into heparinized tubes, the cells were washed and the plasma and buffy coat were removed by centrifugation and resuspension three times in 135 mM NaCl/5 mM sodium phosphate (pH 7.4). The cells were haemolyzed for 15 min in 15 vol. of ice-cold haemolysis medium, 1 mM EDTA/5 mM Tris (pH 7.5) [9]. The ghosts were centrifuged at 16000 $\times g$ for 30 min at 2°C and the supernatant was aspirated. The pellet was washed by centrifugation and resuspension in the haemolysis medium three times and the creamy-white membranes were finally resuspended in 5 mM Tris (pH 7.4) to a volume which was 125–150% of the original volume of blood. In order to ensure maximum exposure of ATPase catalytic sites to the substrates, the membranes were frozen and thawed twice prior to assay [9].

Na^+,K^+-ATPase assay. Na^+,K^+-ATPase was assayed as the difference between the ATPase activity in the absence and the presence of ouabain (0.2 mM). Assays were started by the additions of membranes (100–200 μg protein) to an incubation medium containing 4 mM ATP/4 mM $MgCl_2$/100 mM NaCl/10 mM KCl/0.2 mM EDTA/15 mM Tris (pH 7.4) in a total volume of 1.5 ml. The reaction mixtures were incubated for 30 and 60 min to ensure that the reaction was linear with respect to time at each temperature and the amount of phosphate liberated during the reactions was determined [10]. All protein determinations were measured by the method of Lowry et al. [11].

Influx studies. Fresh venous blood was washed by centrifugation and resuspension three times in a medium containing 145 mM NaCl/10 mM D-glucose/10 mM Tris (pH 7.4) in order to remove the plasma and buffy coat. The erythrocytes were finally resuspended in a medium containing 145 mM NaCl/10 mM D-glucose/25 mM $CaCl_2$/7.5 mM KCl/10 mM Hepes (pH 7.4) and influx of Na^+ or K^+ was measured by the use of ^{22}NaCl or ^{86}RbCl as described by Hall et al. [12].

Briefly, cells were suspended at a haematocrit of 5%, determined by measuring their haemoglobin content with Drabkins reagent [13], in a total volume of 1 ml. Incubations were for 30 min and ouabain-sensitive and furosemide-sensitive components of Na^+ or K^+ influx were measured in the presence of 0.2 mM ouabain or 1 mM furosemide, respectively. The residual passive leak was the ion flux measured in the presence of both inhibitors. Samples (200 μl) were taken and added to a solution (800 μl) containing 106 mM $MgCl_2$/10 mM Tris (pH 7.4) [14] and washed rapidly four times in this medium by centrifugation at room temperature for 30 s in an MSE microcentaur centrifuge. The pellet was lysed with 0.1% (w/v) Triton X-100 (500 μl); 5% (w/v) trichloroacetic acid (500 μl) was then added and the tubes were centrifuged for 2 min at high speed in the MSE microcentaur centrifuge. An aliquot of the supernatant (500 μl) was transferred to a scintillation vial. The amount of ^{86}Rb was measured by the Cerenkov effect in a liquid scintillation spectrometer and the amount of ^{22}Na was determined after addition of a scintillation cocktail (5 ml, Fisofluor 'MPC').

Sodium and potassium determinations. Na^+ and K^+ were measured in dilutions of media and cells with an EEL model 150 clinical flame photometer.

Phospholipid extraction and analysis. Fresh venous blood was collected into heparinized tubes. The cells were washed and the plasma and buffy coat were removed by centrifugation and resuspension three times in 135 mM NaCl/5 mM sodium phosphate (pH 7.4). The packed cells were then extracted by the method of Folch et al. [15]. The lipid extracts were analysed by two-dimensional thin-layer chromatography on silica gel 60 (Merck) containing Florisil (1.7%, w/w Merck) as described previously [16]. The phosphorus content of each spot was determined [17] after visualization by brief exposure to iodine vapour.

Reagents. Ouabain, furosemide, Tris and Hepes were obtained from the Sigma Chemical Com-

pany. Radio isotopes were from Amersham International.

Statistical evaluation. Comparisons of Na^+,K^+-ATPase activities and ion influx rates between the red cells of patient 1 and control red cells were made using an age- and sex-matched control. In the case of comparisons between patient 2 (an affected individual) and patient 3 (an unaffected sister) the difference in age was 2 years. All values were the means of four replicate measurements ± standard deviation. Phospholipid analyses were performed on five control and three affected individuals as shown in Table I. *P* values were derived from Student's *t*-test.

Results

Erythrocytes from affected individuals have an increased temperature-sensitive permeability to K^+

Fig. 1 shows that the concentration of plasma K^+ increases in affected individuals when the whole blood is allowed to stand for various times prior to separation of the erythrocytes from plasma by centrifugation. Furthermore, as reported previously for this syndrome [6,7,8], the leak is exaggerated at lower temperatures. Fig. 2 shows that the genetics of the syndrome are consistent with an autosomal dominant mode of inheritance in this family [6].

Temperature dependence of Na^+,K^+ ATPase activity

Fig. 3 shows that there is no difference in the sensitivity to temperature of the Na^+,K^+-ATPase activity between patient 1 and a control individual. The data are presented as Arrhenius plots and show that the activation energy of the Na^+,K^+-ATPase reaction in affected individuals is identical to that of control cells. Identical data were obtained in a comparison between patient 2 and another control. The data points fall on a straight line for both series of measurements; the correlation coefficient for the control data was 0.992 and for the pseudohyperkalaemic was 0.991.

Ouabain-sensitive K^+ influx

The influx of K^+ through the Na^+,K^+-ATPase was measured as the ouabain-sensitive component of K^+ influx. Fig. 4 shows that, consistent with the

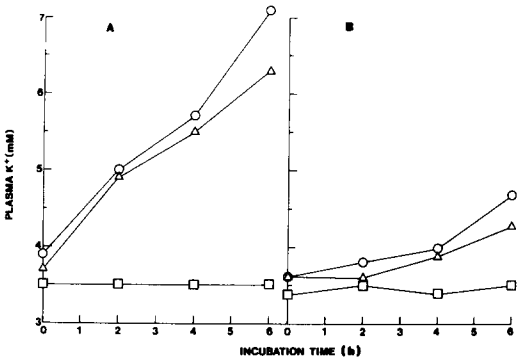

Fig. 1. The effect of temperature on net K^+ efflux from red cells, in whole blood, of controls (□) and two familial pseudohyperkalaemics: ○, patient 1; △, patient 2. A, 21°C; B, 37°C.

results of our study on the effect of decreased temperature on the enzymatic activity of the pump (Fig. 3), we found no difference between the temperature dependence of K^+ influx via the pump between patient 1 and a control. Patient 2 and her unaffected sister (patient 3) were also compared and identical values were obtained. We concluded from these experiments that the increased erythrocyte permeability to K^+ at lower temperatures in pseudohyperkalaemics was not due to a defect in the pump.

Furosemide-sensitive K^+ influx

Flux of K^+ through the Na^+,K^+-cotransport system was measured by its sensitivity to inhibition by the loop diuretic, furosemide. Fig. 5 dem-

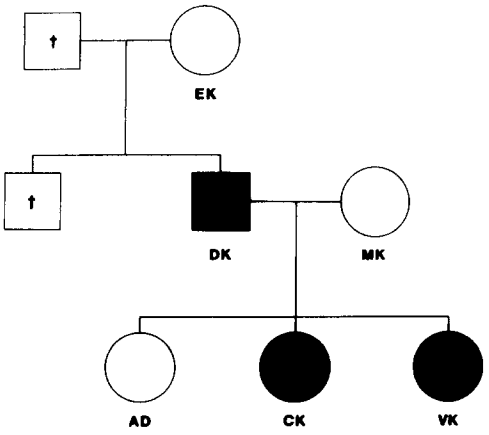

Fig. 2. The incidence of familial pseudohyperkalaemia in the family studied. Shaded individuals are affected; †, deceased. DK, patient 1; VK, patient 2; AD, patient 3.

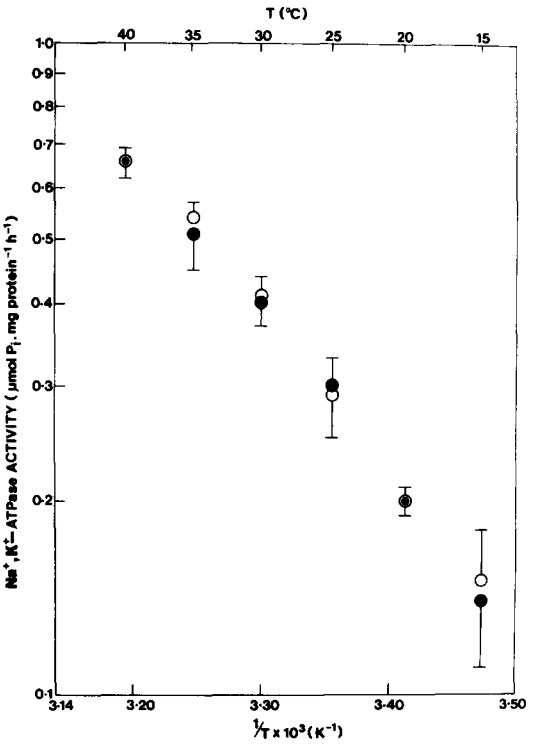

Fig. 3. An Arrhenius plot of the effect of temperature on the activity of the Na^+,K^+-ATPase. The reciprocal of the absolute temperature is plotted against logarithm to the base 10 of the enzymic activity for a control (●), and patient 1 (○).

onstrates that the cotransport function of patient 1 and a control behave similarly down to a temperature of 25°C. However, below 25°C, it is clear that cotransport activity in the affected individual is decreased relative to the control.

We observed a similar temperature sensitivity of the cotransport system of patient 2 when compared with her unaffected sister patient 3 whose influx values were exactly the same as the other control. One possible explanation for this observed difference might have been that we had not succeeded in inhibiting all the cotransport activity in the affected individuals. We therefore prepared a dose-reponse curve at 31°C for both patient 1 and a control and found that maximal inhibition was obtained in both cases with a concentration of 1 mM furosemide. Stewart et al. [18] have shown that in normal red cells the ability of 1 mM furosemide to inhibit cotransport is not reduced at low temperatures. We concluded that either the membrane proteins responsible for cotransport were in some way abnormal or that the membrane environment might be altered in such a way as to compromise their function in the affected cells at lower temperatures.

Furosemide-sensitive Na^+ influx

Garay et al. [19] have shown that Na^+,K^+-cotransport in human red cells has a 1 : 1

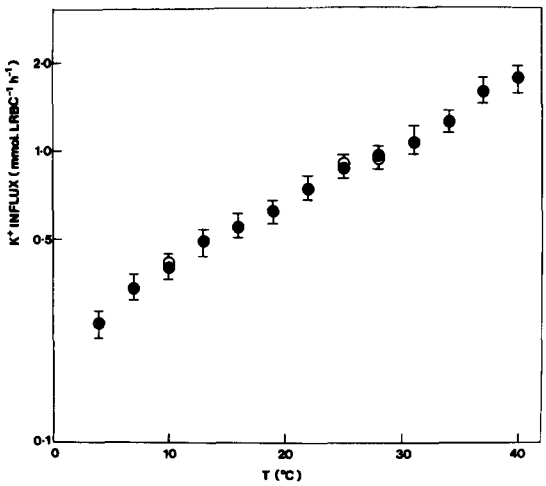

Fig. 4. The effect of temperature on the ouabain-sensitive component of K^+ influx for a control (●), and patient 1 (○). LRBC, litre red blood cells.

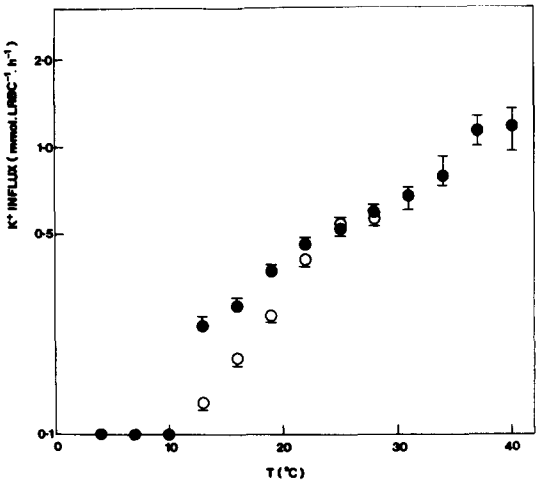

Fig. 5. The effect of temperature on K^+ influx via Na^+,K^+-cotransport for a control (●), and patient 1 (○). LRBC, litre red blood cells.

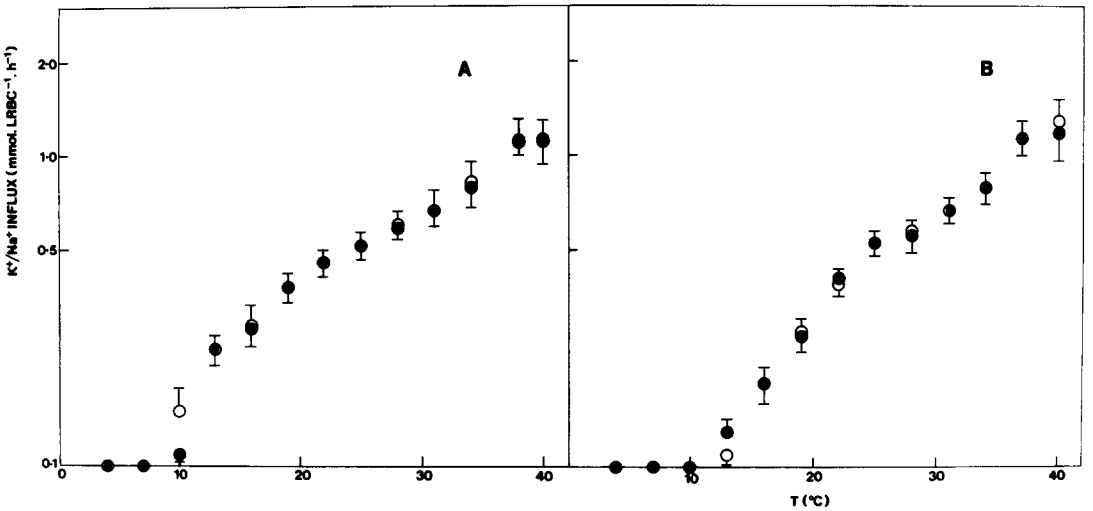

Fig. 6. A comparison of Na^+ (○) and K^+ (●) influx via the furosemide-sensitive component as a function of temperature: A, control; B, patient 1. LRBC, litre red blood cells.

stoichiometry. In order to confirm that we were observing an effect on cotransport, we compared the effect of temperature on furosemide-sensitive Na^+-transport with K^+-transport. Fig. 6 shows that in both a control (A), and patient 1 (B), Na^+ influx that is inhibitable by furosemide has the same rate as K^+ influx throughout the temperature range studied; we concluded that we were indeed identifying an alteration in the cotransport component of K^+ flux.

Residual passive permeability to K^+

The residual passive leak was the K^+ flux that was not inhibited by both ouabain and furosemide. Fig. 7 confirms the observation by Stewart et al. [18] that the passive permeability of normal human red cells shows a minimum around 15°C.

The effect of temperature on the passive K^+ leak in a pseudohyperkalaemic (patient 1) is even more marked. Indeed, at 4°C the passive permeability of the affected cells is greater than that at 37°C. The residual passive permeability of the red cells of patient 3 showed an identical temperature dependence to the other non-related control, whereas the values for her affected sister patient 2 were similar to those of patient 1.

Phospholipid composition of erythrocyte membranes

An association between increased potassium permeability and an increased phosphatidylcholine content of human erythrocytes has been observed previously [4,20]. Furthermore, comparisons between different species have suggested a relation-

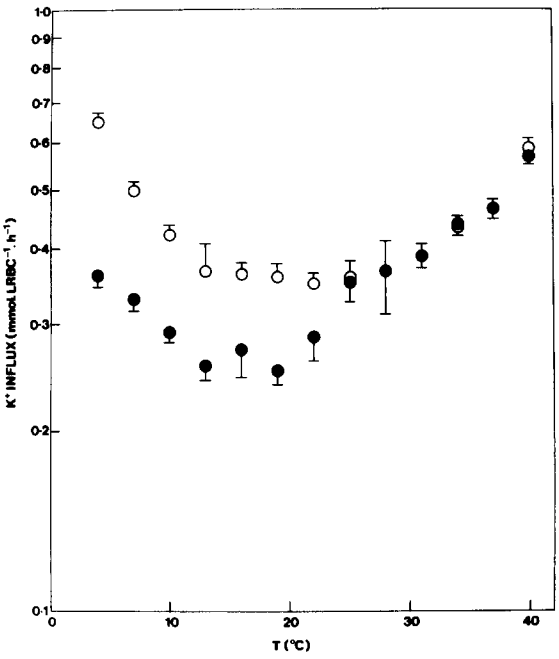

Fig. 7. The effect of temperature on the ouabain- and furosemide-resistant, passive leak component of K^+ influx for a control (●), and patient 1 (○). LRBC, litre red blood cells.

TABLE I

PHOSPHOLIPID COMPOSITION OF RED CELLS FROM CONTROL AND PSEUDOHYPERKALAEMIC INDIVIDUALS

Red cells were extracted and their phospholipid contents were analyzed by two-dimensional thin-layer chromatography as described in the text. The control values represent the means ± S.E. for five individuals. Each analysis was conducted in quadruplicate. The values for the pseudohyperkalaemic individuals are the means ± S.E. of quadruplicate analyses in three affected people. *P* values are derived from Student's *t*-test.

Phospholipid	mol% of total phospholipid		
	control	pseudohyperkalaemic	*P*
Phosphatidylcholine	29.8 ± 0.2	33.3 ± 0.3	0.010
Sphingomyelin	28.8 ± 0.2	27.6 ± 0.9	> 0.05
Phosphatidylethanolamine	24.4 ± 0.3	22.7 ± 0.2	0.007
Phosphatidylserine	11.2 ± 0.5	12.4 ± 0.7	> 0.05
Phosphatidylinositol	3.5 ± 0.3	3.7 ± 0.4	> 0.05
Lysophosphatidylcholine	1.4 ± 0.4	1.1 ± 0.4	> 0.05
Phosphatidic acid	1.0 ± 0.4	0.6 ± 0.1	> 0.05

ship between the phosphatidylcholine content of red cell membranes and their permeability to potassium ions [21,22]. We wondered whether an altered phosphatidylcholine content might help to explain the abnormal temperature dependency of potassium flux through both the cotransport and residual passive permeability routes in pseudohyperkalaemic erythrocytes.

The results for the analyses performed on five control and three affected individuals are shown in Table I. The controls included one unaffected person from the same family as the affected individuals. There is clearly a significantly higher amount of phosphatidylcholine in the red cell membranes from pseudohyperkalaemics. There is also an apparent compensatory decrease in the amount of phosphatidylethanolamine.

Conclusions

The temperature dependencies of the three major components of K^+ flux in normal human red cells reported here are very similar to those described by Hall et al. [12].

We have identified cotransport and the passive permeability of K^+ as the components of K^+ flux that show an abnormal response to decreased temperature in the red cells of pseudohyperkalaemics. To our knowledge, there have been only two other studies on the nature of the K^+ leak in families with a similar syndrome [23,24]. In the former study the affected component of K^+ flux was found to be the residual K^+ permeability, but there was a compensatory increase in the activity of the pump. Similar results were obtained in the latter study. It therefore seems most probable that there is more than one familial pseudohyperkalaemia syndrome. The nature of the membrane defect which might affect both the cotransport and passive permeability of K^+ remains to be elucidated. It seems possible that an altered membrane lipid environment could account for the fact that both routes of K^+ flux are affected. The results from this study are consistent with the hypothesis that an increased amount of phosphatidylcholine is associated with an elevated permeability of the red cell membrane to potassium ions. Phospholipids in the red cell membrane are asymmetrically distributed between the two halves of the bilayer, with the majority of phosphatidylcholine present in the outer half [21]. It would be intriguing to learn whether the increased amounts of phosphatidylcholine in the cells from pseudohyperkalaemics are distributed evenly between the two halves of the bilayer. If the increase is restricted to the outer bilayer, which contains about 75% of the total phosphatidylcholine, the percentage increase in the amount of this phospholipid in the outer monolayer in pseudohyperkalaemics becomes even more marked.

Acknowledgements

We would like to thank the Scottish Home and Health Department for the financial support of this work. We would also like to thank the patients for their enthusiastic co-operation, without which this study would have been impossible.

References

1 Glynn, I.M. (1957) Prog. Biophys. Biophys. Chem. 8, 241–307
2 Wiley, J.S. and Cooper, R.A. (1974) J. Clin. Invest. 53, 745–755
3 Lauf, P.R. and Joiner, C.H. (1976) Blood 48, 457–468
4 Wiley, J.S., Ellory, J.C., Shuman, M.A., Shaller, C.C. and Cooper, R.A. (1975) Blood 46, 337–356
5 Gunn, R.B., Silvers, D.N. and Resse, W.F. (1972) J. Clin. Invest. 51, 1043–1050
6 Stewart, G.W., Fyffe, J.A., Corrall, R.J.M., Stockdill, G. and Strong, J.A. (1979) Lancet ii, 175–177
7 Luciani, J-C., Lavabre-Bertrand, F., Fourcase, J., Barjon, P., Mimran, A. and Callis, A. (1980) Lancet i, 491
8 Leadbetter, S. and O'Dowd, T.C. (1982) Lancet ii, 103–104
9 Sigström, L. and Waldenström, J. (1980) Clin. Chim. Acta 104, 345–348
10 Petersen, G.L. (1978) Anal. Biochem. 84, 164–172
11 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
12 Hall, A.C., Ellory, J.C. and Klein, R.A. (1982) J. Membrane Biol. 68, 47–56
13 Catton, W.T. (1957) Physical Methods in Physiology, Pitman, London
14 Dunham, P.B. and Ellory, J.C. (1980) J. Physiol. (London) 307, 25–38
15 Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–508
16 McKenzie, R.C. and Brophy, P.J. (1984) Biochim. Biophys. Acta 769, 357–508
17 Rouser, G., Fleischer, S. and Yamamoto, A. (1970) Lipids 5, 5494–5496
18 Stewart, G.W., Ellory, J.C., and Klein, R.A. (1980) Nature (London). 286, 403–404
19 Garay, R., Adragna, N., Canessa, M. and Tosteson, D. (1981) J. Membrane Biol. 62, 169–174
20 Shoket, S.B., Livermore, B.M. Nathan, D.G. and Jaffé, E.R. (1971) Blood 38, 445–456
21 Deuticke, B., Grunze, M. and Haest, C.W. (1980) in Membrane Transport in Erythrocytes (Lassen, U.V., Ussing, H.H. and Wieth, J.O., eds.) pp. 143–160, Munksgaard, Copenhagen
22 Kirk, R.G. (1977) Biochim. Biophys. Acta 464, 157–164
23 Enet-Renou, N., Mirouze, J., Dagher, G., Dupont, M., Richard, J.L. and Mimran, A. (1983) Sem. Hop. Paris 59, 3121–3126
24 Ellory, J.C. and Stewart, G.W. (1984) J. Physiol. (London) 346, 83p