the fish revived subsequently in air-saturated water, it became evident that the anaerobic ability to survive hypoxia is greater in *C. auratus* than in *T. mossambica, P. sarana* and *R. corsula.* However, only observations at other temperatures can give the full extent of temperature influence on the hypoxic tolerance of the fish. When oxygen was being reduced in a respirometer, some fish were seen to become more passive and some more active, thereby establishing a dichotomy in behavior of fishes at low oxygen levels[10,13]. The passive fish (e.g. *T. mossambica*[10] and *Chanos chanos*[13]) are also likely to escape hypoxic waters and reach oxygen-rich water if available. The full capacity for low oxygen tolerance can perhaps be known only if the fishes concerned are studied after acclimating them to low oxygen. But in the few known cases, low oxygen acclimation increased blood oxygen capacity and also efficiency in the utilization of oxygen, but not oxygen tolerance and anaerobic capacity[6,14,15].

1 From a thesis (M.P.M.) approved for the degree of Doctor of Philosophy, Madurai Kamaraj University, Madurai, India.
2 We wish to express our thanks to Prof. S. Krishnaswamy for providing facilities and to Dr S.V. Job for suggestions.
3 P. Blazka, Physiol. Zool. *31,* 117 (1958).
4 G.B. Mathur, Nature *214,* 318 (1967).
5 C.W. Coulter, Nature *215,* 317 (1967).
6 M.N. Kutty, J. Fish. Res. Bd Can. *25,* 1689 (1968).
7 M. Peer Mohamed, Ph.D. thesis, Madurai Kamaraj University, Madurai, India 1974.
8 M.N. Kutty and M. Peer Mohamed, Aquaculture *5,* 253 (1975).
9 M. Peer Mohamed and M.N. Kutty, Hydrobiologia *76,* 3 (1981).
10 M. Peer Mohamed and M.N. Kutty, Proc. Indian Acad. Sci. *89,* 537 (1980).
11 M.N. Kutty, M. Peer Mohamed, K. Thiagarajan and A.N. Leonard, Indian J. exp. Biol. *9,* 218 (1971).
12 American Public Health Association, ed., Standard methods for the examination of water and wastewater, 12th edn. A.P.H.A. Inc., New York 1965.
13 A.K.M.S. Hamsa and M.N. Kutty, Indian J. Fish. *19,* 76 (1972).
14 C.L. Prosser, B.R.A. Pinc and C.Y. Lerner, Physiol. Zool. *30,* 137 (1957).
15 M.P. Shepard, J. Fish. Res. Bd Can. *12,* 387 (1955).

Voltage transients during ionic substitution in renal cortical tubules[1]

A.G. Lopes, G.B. de Mello and G. Malnic[2]

Department of Physiology, Instituto de Ciências Biomédicas, Universidade de São Paulo, C.P. 4365, 01000 São Paulo (Brazil), 11 September 1981

Summary. A voltage transient is described which is found during proximal tubular perfusion with impermeant cation or anion salt solutions in the rat. It was shown that the magnitude of transepithelial diffusion potentials depended on luminal hydrostatic pressure, suggesting that the observed transients might be the consequence of the enlargement of ionic pathways by tubular dilatation. Thus, when reporting PD values, care should be taken to define the pressure levels at which measurements were performed.

When studying the transepithelial PD (potential difference) in the mammalian proximal tubule, it is important to ascertain that the microelectrode tip is within the lumen when measurements are made[3]. One of the methods used for this localization is luminal perfusion with impermeant cations. Since the paracellular pathway is the main passive ion path across the epithelium, ion concentration gradients across the epithelium generate diffusion potentials. The proximal tubule is highly permeable both to Na and Cl[4]. When Na is replaced by a chloride salt of a less permeant cation, like choline or magnesium, the tubular lumen is rendered positive due to the Na concentration gradient that is established. This potential shift is obviously detected only when the microelectrode tip is correctly localized in the tubular lumen. In an earlier paper we studied proximal transtubular PD in the rat using this localization method[5]. In these studies, we noted a positive voltage transient at the start of perfusion with isosmotic choline chloride solutions that has not been reported before. The present paper analyses the origin of this voltage transient.

Material and methods. Studies were performed on male rats weighing between 250 and 350 g. Anesthesia was induced by i.p. injection of pentobarbitone (40 mg/kg b.wt). The rats received an infusion of 3% mannitol in saline at 0.08 ml/min during the experiments. The animal preparation as well as the micropuncture and electrical measurement techniques used have been described previously[5]. The microelectrodes used were: a) Single and double-barrelled Ling-Gerard microelectrodes with tip diameter less than 0.5 µm filled by boiling with 3 M KCl solution to which

Transepithelial PD and voltage transients during luminal microperfusion of rat proximal tubule

Perfusion	Microelectrodes	PD max (mV)	PD st (mV)	△PD (mV)	n
Choline Cl iso	Ling-Gerard	16.8±0.79	9.9±0.79	6.9±0.50*	61
Choline Cl iso	Ringer-agar	14.3±0.41	9.4±0.42	4.9±0.23*	117
Choline Cl 15 mM	Ringer-agar	-	0.66±0.21	-	19
$MgCl_2$	Ringer-agar	8.0±0.44	4.4±0.43	3.6±0.23*	38
Na_2SO_4	Ringer-agar	−5.8±0.19	−1.6±0.13	−4.2±0.22*	58
Sodium cyclamate	Ringer-agar	−7.2±0.50	−4.7±0.38	−2.5±0.26*	18

PD max, peak value of transepithelial voltage including transient; PD st, PD during continuous perfusions; △PD, value of the voltage transient (PD max - PD st); Sign (+/−) denotes luminal polarity. *$p < 0.01$ (difference with $\Delta = 0$). n, number of perfusions.

fluorescein was added. b) Micropipettes with 3–5 µm tip diameter, filled with a solution whose main ionic components were similar to those of proximal tubular fluid, plus 2% agar[5]. Microelectrodes with tip potential less than 5 mV and resistances of 5–20 MΩ were used. PDs were measured by a Keithley mod. 602 or 615 electrometer and recorded by Beckman mod. RP Dynograph. Tubular effective resistances were measured by passing current from a Harvard mod. 245 stimulator through one barrel of a microelectrode, PD changes being measured by the other. Specific resistances were obtained by cable analysis[5]. The intratubular hydrostatic pressure was measured by means of the Landis technique[6]. Tubular diameters were measured with a Bausch and Lomb ocular filar micrometer. Tubules were perfused with single or double barrelled micropipettes filled with lissamine green (0.05%) coloured solutions. The perfusion solutions contained 150 mM of one of the following salts: NaCl, $MgCl_2$, Na_2SO_4, choline chloride and sodium cyclamate. A solution with 135 mM NaCl plus 15 mM choline chloride was also used. In some experiments, atropine (40 µg/ml), gallamine (Flaxedil) (100 µg/ml), 10^{-3} M furosemide or 10^{-3} M NaCN, were added to the choline chloride solution.

Results. The voltage transient observed during perfusion of proximal tubules with isotonic (150 mM) choline chloride is shown in figure 1. Immediately upon starting the perfusion a positive deflection of $16.9 \pm 0.79(61)$ mV (using Ling-Gerard microelectrodes) and of $14.3 \pm 0.41(117)$ mV (using Ringer-agar-filled micropipettes) was recorded. This value then fell with a half time of 1.42 ± 0.10 sec ($n = 30$) to a steady level maintained during the rest of the perfusion. The value of this level was of the order of 9–10 mV (table). To investigate whether this voltage transient might be a consequence of a pharmacological action of choline, perfusions with 15 mM choline chloride/135 mM NaCl were performed. As shown in the table, no significant voltage deflection was found. Similarly, when metabolic and transport inhibitors (NaCN, furosemide) or anticholinergic drugs (atropine, gallamine) were added to choline chloride or NaCl solutions, no changes in the voltage tracings were observed. The table also shows that voltage transients were found not only with choline perfusions, but also with $MgCl_2$, Na_2SO_4 and sodium cyclamate. In the case of the two latter, lumen negative voltages and transients were obtained, indicating that such transients can be found independently of the nature of the impermeant ion used.

During the experiments described, it was noted that the magnitude of the observed voltage in some perfusions was dependent on the rate of perfusion; when high perfusion rates were used, the measured voltage tended to decrease. This observation could imply that an increased tubular pressure distended the tubule and thereby decreased the transepithelial voltage. This possibility was studied by measuring simultaneously tubular hydrostatic pressure by the Landis technique and PD, during perfusion at different rates produced by varying pressures applied to the perfusion pipette by means of a mercury column. Figure 2A shows that as perfusion rate (and consequently tubular pressure) is increased, there is an initial rise of the observed PD, probably consequent to a more efficient substitution of the tubular NaCl by choline chloride. However, increasing pressure further leads to a marked reduction in voltage. Transpithelial resistances follow an approximately similar pattern, as is shown in figure 2B.

Discussion. The observed voltage transients do not appear to be caused by microelectrode artifacts, since very similar results were obtained when Ling-Gerard microelectrodes or Ringer-agar-filled micropipettes were used. It has been shown that it is possible to induce action-potential-like voltage transients in epithelia[7] and even in lipid bilayers[8]. However, the voltage transient described in this report does not seem to be due to a pharmacologic effect of choline, since luminal perfusion with 15 mM choline chloride completed to isotonicity with NaCl did not induce such a transient (see table), and other drugs of different pharmacologic action did not alter these tracings. The perfusion of proximal tubules with a number of impermeant ions, both anions and cations, also leads to voltage transients. Furthermore, inhibition of salt transport by NaCN or furosemide, which have a definite effect on NaCl transfer in this structure[9], also did not alter the nature of the transient. These observations suggest that the voltage tracings found during perfusions with impermeant ions, including the initial transients, are a function of the establishment of diffusion potentials, independent of active transport mechanisms. However, this conclusion still does not explain the origin of the transient voltage displacements. Figure 2 shows that these appear to be related to tubular pressure. A perfusion rate that leads to extensive substitution of the normal luminal salts appears to require pressures considerably in excess of those normally found in these tubular segments, which are in the range of 8.5–21.5 mm Hg[10]. Pressures like those given in figure 2 can be obtained during luminal perfusions driven by hand-pressed syringes. Tubular diameters increase considerably during such perfusion: control values were 22.0 ± 1.04 µm ($n = 9$), and values observed during perfusion (perfusion pressure higher than 30 mm Hg) were 31.7 ± 0.97 µm ($n = 25$). These data suggest that the distension of the tubular wall, possibly leading to enlargement of

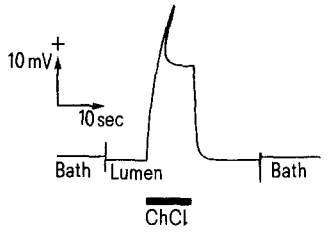

Figure 1. Representative example of voltage profile during proximal tubular perfusion with isotonic choline chloride (ChCl).

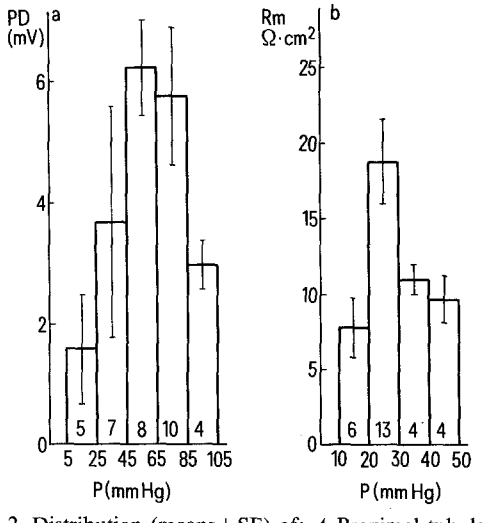

Figure 2. Distribution (means ± SE) of: *A* Proximal tubular transepithelial potential difference (PD) and *B* specific resistance (Rm) according to intraluminal hydrostatic pressure (P). Numbers within columns represent number of observations.

the paracellular pathway, may play a role in the fall of the transepithelial PD to the steady level given in the table. Thus, it appears that the value corresponding to the normal situation of the epithelium may be the peak value of the transient, while the steady level corresponds to a situation where an increased diameter of the transepithelial path increases the distance between fixed charges on its walls, as proposed by Sollner[13], thereby reducing the ionic selectivity of this barrier. This view is supported by resistance measurements (fig. 2B), which show first an increase with pressure, probably due to the lower epithelial conductance to choline in relation to sodium. Their fall at higher pressures may be due to enlargement of the ionic path through the epithelium. The present interpretation is compatible with the findings of Grandchamp and Boulpaep, who found increased transepithelial NaCl fluxes when luminal pressure was increased in *Necturus*[11]. On the other hand, Maunsbach and Boulpaep[12] found decreased interspace width and increased resistance when luminal pressure was increased in *Necturus*. However, these increased pressures were much lower than the maximal values used in the present study, so that their data correspond to the ascending part of the curve of figure 2. In conclusion, we believe that alterations in luminal pressure may affect transepithelial PD during luminal perfusion in proximal tubules to an important extent. Thus, in presenting data on the effects of luminal perfusion on transepithelial PD, the pressure levels at which they were obtained should be carefully defined.

1 Supported by Fund. de Amparo a Pesquisa do Estado de São Paulo, and FINEP.
2 To whom reprint requests should be addressed.
3 E. Frömter, T. Wick and U. Hegel, Pflügers Arch. *295*, 365 (1967).
4 E.L. Boulpaep, Kidney Int. *9*, 88 (1976).
5 G.B. de Mello, A.G. Lopes and G. Malnic, J. Physiol., Lond. *260*, 553 (1976).
6 E.M. Landis, Am. J. Physiol. *75*, 548 (1926).
7 A. Finkelstein, J. gen. Physiol. *47*, 545 (1964).
8 P. Mueller and D.O. Rudin, Nature *217*, 713 (1968).
9 G. Malnic, H. Enokibara, M. Mello Aires, and F. Lacaz-Vieira, Pflügers Arch. *309*, 21 (1969).
10 M.E.M. Allison, E.M. Lipham and C.W. Gottschalk, Am. J. Physiol. *223*, 975 (1972).
11 A. Grandchamp and E.L. Boulpaep, J. clin. Invest. *54*, 69 (1974).
12 A.B. Maunsbach and E.L. Boulpaep, Kidney Int. *17*, 732 (1980).
13 K. Sollner, in: Electrochemistry in biology and medicine, p. 33. Ed. T. Shedlovsky. Wiley, New York 1955.

Enzyme activities of a cold-resistant L cell variant

E. Holečková, V. Mandys, M. Elleder and B. Jakoubek

Institute of Physiology, Czechoslovak Academy of Sciences, Kidenská 1083, 142 20 Prague (Czechoslovakia), and Ist Department of Pathology, Charles University, Prague (Czechoslovakia), 3 August 1981

Summary. Resistance of mouse L cells to cold increased the activity of nonspecific esterase, acid phosphatase and adenosine triphosphatase, but did not influence the activity of succinate dehydrogenase.

Cold-resistant, heat-sensitive L cell variants isolated in our laboratory differed from their parent populations by a more efficient regulation of the intracellular concentration of K^+ ions at 4 °C, by a higher O_2 consumption at 30 and 36 °C and by a higher activity of cytochrome oxidase together with a higher sensitivity to KCN at 30 and 36 °C. Therefore, the activity of ATPase involved in the transport of potassium ions and the activity of succinate dehydrogenase involved in cellular oxidations was compared in the parent L-As cells and their cold-resistant LC3 variant. Acid phosphatase and nonspecific esterase were included in the comparison in view of the findings suggesting the role played by the lysosomal enzymes and some esters in cold acclimation and thermogenesis in the brown fat of the rat[4,5].

Materials and methods. Parent L-As and variant LC3 cells[6] were grown for 3 weeks at 36 °C in Eagle's MEM with 2% FCS and 40 µg/ml gentamycine. Then, they were cultured for 4 days in 5% CO_2 atmosphere on cover slips in Petri dishes or on semipermeable dialyzing membranes (Cuprophan, Wuppertal, FRG) in special chambers as described previously[7,8]. After decantation of the medium, they were rinsed 3 times with PBS, dried and used for histochemistry. Acid phosphatase was demonstrated by simultaneous azocoupling (Naphtol-ASBI derivatives, hexazonium-p-rosaniline) in cultures on semipermeable membranes. Nonspecific esterase (α-naphtyl acetate, hexazonium-p-rosaniline), ATPase (Naphtol-AS phosphate, Fast Blue BB) and succinate dehydrogenase (SDH - acceptor NBT) were demonstrated in cultures grown on cover slips. Densitometrical evaluation of histochemical reactions was performed with a Schnellphotometer II, Zeiss, Jena, GDR, in 25–40 fields (circular areas, diameter 2 mm, objective × 6) of the preparations, and in 30 randomly chosen individual cells (objective × 40) of each preparation. Significance of the differences between the 2 populations was evaluated with Student's t-test.

Results. The figure shows the intensity and localization of enzyme activities in the parent L-As and the variant LC3 cells. The activity of nonspecific esterase is strikingly high in the LC3 cells, especially in the large polykaryons and giant mononuclears characteristic for the variant, where the intensive reaction covers all intracellular structures (fig. a). Mononuclear cells have negative zones over the nuclei similar to those of the L-As cells, shown on (fig. b). Also the activity of acid phosphatase occupies in the variant cells a larger area of cytoplasm (fig. c) than in the parent cells with predominantly juxtanuclear localization (fig. d). The activity of ATPase is again increased in the variant cells; it is localized in the cytoplasm and on cell surfaces (fig. e). The surface localization is lacking in the L-As cells (fig. f). The 2 populations do not differ in the activity of succinate dehydrogenase (figs g and h). The table presents the validity of the differences seen microscopically after densitometrical quantitation. The activity of all enzymes except dehydrogenase is significantly higher in the variant than in the parent cells, and the significance increases when values obtained in individual cells are compared.

Discussion. Our cold-resistant L cell variants selected from heterogenetic parent populations live at 4 °C for years with only short intermissions at 36 °C for multiplication and passage, and they bear some traits typical for specialized tis-