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.
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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

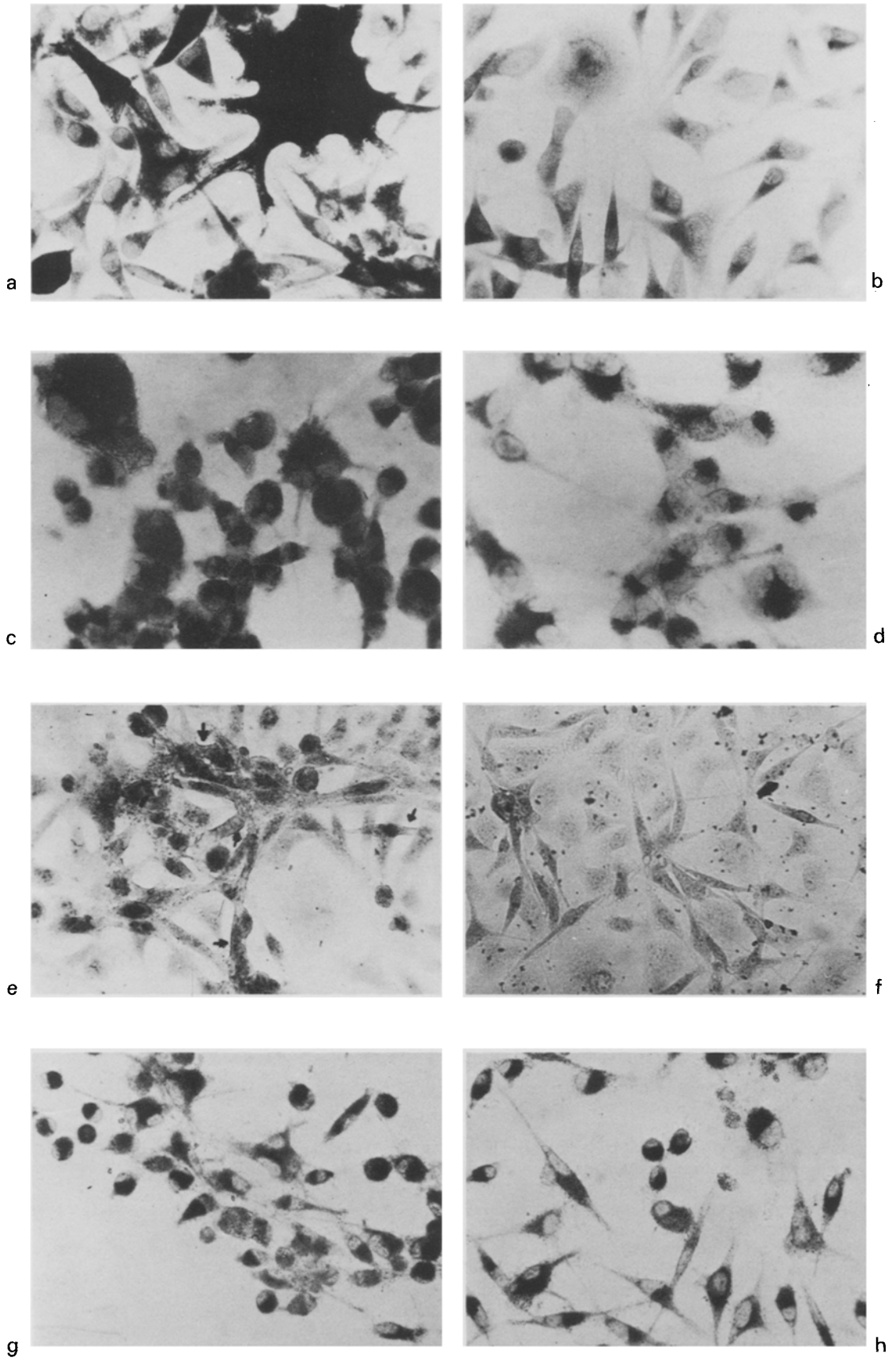
Summary. Resistance of mouse L cells to cold increased the activity of nonspecific esterase, acid phosphatase and adenosine triphosphatase, but did not influence the activity of succinate dehydrogenase.

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-



Activity of nonspecific esterase (a and b), acid phosphatase (c and d), adenosine triphosphatase (e and f) and succinate dehydrogenase (g and h) in the cold-resistant LC3 (a, c, e and g) and the parent L-As cells (b, d, f and h). Histochemical reactions without counterstain, magnification × 320. Arrows indicate ATPase activity on the surfaces of LC3 cells.

Mean ± SE of enzyme activities measured in circular areas of the preparations, objective × 6 (indicator: intensity of staining expressed as optical density) in the cold-resistant LC3 and the parent L-As cells

Enzyme	Cells LC3 Mean ± SE	No. of fields	L-As Mean ± SE	No. of fields	Level of significance
Nonspecific esterase	90.85 ± 1.47	28	66.33 ± 0.65	25	0.1%
Acid phosphatase	94.82 ± 1.30	30	83.45 ± 1.39	30	0.1%
Adenosine triphosphatase	63.82 ± 0.16	40	59.20 ± 0.24	40	0.1%
Succinate dehydrogenase	79.80 ± 0.49	30	80.30 ± 0.76	30	NS
The same measured in 30 individual cells (objective × 40)					
Nonspecific esterase	146.86 ± 1.22		132.60 ± 0.85		0.001%
Acid phosphatase	130.70 ± 0.52		127.36 ± 0.53		0.001%
Adenosine triphosphatase	132.06 ± 0.96		123.20 ± 0.45		0.001%
Succinate dehydrogenase	139.50 ± 1.09		139.06 ± 1.44		NS

sues of cold-adapted animals in situ. These characteristics are not lost after many generations at 36 °C, and a comparison of some of their enzyme activities was therefore possible at this temperature where both cell types live undamaged.

The higher activity of ATPase and its localization on cell surfaces in the variant, expected in view of the better regulation of the intracellular K^+ concentration in the cold, confirmed changes in the constitution and function of the surfaces membranes detected also by direct potassium measurements[1], by scanning electron microscopy[9] and by microcinematography[10]. Further studies are necessary to distinguish precisely between the ATPase activity localized on cell surfaces and in the mitochondria. The increased activity of acid phosphatase as well as of other lysosomal enzymes[11] strengthens the hypothesis that lysosomes may be involved in cold adaptation[4]. The nonspecific esterase covers a wide field of reactions; esters of acyl-coenzyme A were suggested as important substrates for cellular respiration and thermogenesis[5], and our preliminary experiments showed also differences between the 2 cell types in the content of cholesterol esters. The lack of difference in the activity of succinate dehydrogenase found in repeated time-independent tests indicates either that this system is not activated at 36 °C in the LC3 cells, or that it is not involved in cold resistance.

Although it is not possible to see a direct analogy between the cold-adapted animal and the cold-resistant cell, yet such cells may offer a valuable model of some processes occuring in situ.

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Effects of denervation and local 6-hydroxydopamine injection on testicular growth in rats

K. Nagai, S. Murano, Y. Minokoshi, H. Okuda and M. Kinutani

Department of Medical Biochemistry and Department of Anatomy, Ehime University Schoool of Medicine, Shigenobu, Onsen-gun, Ehime (Japan 791-02), 26 June 1981

Summary. Denervation and local 6-hydroxydopamine injection on day 13 caused a decrease in the testicular weight by days 42 to 70, which was due primarily to a disturbance in the growth of seminiferous tubules.

Although many reports have documented the important role of the hypothalamo-hypophyseo-gonadal axis in testicular growth and function, little is known about the role of the nervous system. Kuntz[1] and Takahashi[2] reported degeneration and reduction of the germinal epithelium after removal of the sympathetic nerves innervating the testis in the dog and the guinea-pig, respectively. Other workers reported similar findings in the cat[3] and the rat[4], and the reduction of spermatogenesis after similar procedures in man[5–7]. Thus we examined the effects of denervation and local 6-hydroxydopamine (6OHDA) injection on the developmental growth of the testis in the rat, in order to elucidate the neural role.

Materials and methods. Male Wistar strain rats were anesthetized with ether, and 2-mm segments of the right superior and middle spermatic nerves were resected at a distance of 5 mm from the right testes at the age of 13, 19 or 21 days (days 13, 19 or 21; denervation experiments). 6-OHDA hydrobromide (16 µg/g b.wt) in 8 µl of 0.01% ascorbic acid solution was injected beneath the capsule of the right testis on days 13, 17, 21 and 26, whereas the same amount of ascorbic acid solution was injected into the left