differed genetically, as did the number of nestboxes present. Large variations in peak densities of freely growing confined populations of small rodents raised under identical conditions have been observed earlier[3].

Confined populations of small mammals have been extensively used to study fundamental mechanisms of population regulation. The Norwegian lemming has been investigated by De Koch and Rohn[4], but the populations were provided with increased living area and were also disturbed by external factors and illness, making comparisons difficult. Clough[5] found that his confined populations of captive lemmings reached a near-steady level after 3–4 months, but his colonies were kept in smaller pens. The only case known to us where confined rodent populations behaved in a similar way to our lemming colonies is the population of nutria in the Philadelphia Zoological Gardens. The period was, in that case, 12 years[6].

In our experiments decline was caused by a combination of a reduction in the number of pregnant females and increased juvenile and adult mortality (figure 1). In fact, at comparable population densities, infant mortality was higher in the declining than in the increasing population, as evidenced by reduced life expectancy (figure 2). Animals born when the increasing population had 11–20 individuals in experiment 1, had an average life expectancy of 154 days compared to only 10 days for individuals born during the declining population phase. Increased density and eventual decline were characterized by an increase in the general activity, fighting, communal nesting and huddling. As in other studies, these changes in behavior were associated with severe juvenile mortality. Periods of increased infant mortality were associated with retarded growth, and the individuals which died shortly after weaning (i.e. between 2–3 weeks of age) showed signs of faltering already when 5–10 days old, when they were completely dependent upon the mother for milk and care. These effects may be caused by prenatal influences[7], maternal neglect and lactation failure[8], as well as the effects of huddling and overcrowding in the nest boxes[3]. Physiological changes, such as retarded growth, may persist in individuals for months after a population peak, a feature which has been shown in other rodents such as California voles[9] and in rabbits[10].

Part of the pen was occupied by more or less isolated, probably territorial, individuals, whereas the remainder was occupied by the subordinates. Huddling in nestboxes increased during the peak and decline phases and some nestboxes could house more than 20 adults. Hence, huddling had a detrimental effect on juvenile survival.

Our confined populations represent unnatural situations, especially since emigration of individuals was prevented. The results demonstrate, however, that even confined lemming populations, under controlled conditions, exhibit strong fluctuations in numbers. It was obvious that population parameters, such as infant mortality, are not simple functions of population density, but are dependent upon the population phase, i.e. whether increasing or declining.

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Potassium uptake by cold-resistant mouse L cells stored at low temperature

E. Holečková and N. Kříž

Institute of Physiology, Czechoslovak Academy of Sciences, Vídeňská 1083, 14220 Prague (Czechoslovakia), 21 February 1979

Summary. Adaptation of a cultured mouse L cell population to 4 °C increased the survival of the cells and induced the uptake of potassium from the medium at this temperature.

Accumulation of intracellular potassium is known to occur not only in neurons, but also in other cell types before DNA synthesis and mitosis[1,2]. Intracellular accumulation of K^+ was also described in cold-stored cells and tissues of hibernators[3]. Previous experiments with cultured mouse s.c. fibroblasts (L cells) demonstrated an increased retention of K^+ in cold-stored sublines previously adapted to 4 °C, compared with that in unadapted parent populations[4]. Some findings even suggested the possibility of K^+ uptake in cold-stored, cold-adapted cells. To verify this possibility, we studied the changes in the intracellular K^+ concentration ($[K_i^+]$) in cold-stored LC3 cells, selected for cold resistance by repeated cooling of the parent L cell population[5]. The $[K_i^+]$ changes were estimated indirectly by measuring the K^+ concentration in the medium ($[K_e^+]$) of the closed culture system with ion-selective microelectrodes (ISMs) without disturbing the cells.

Materials and methods. A subline of the L cells called L-As and its cold-resistant derivative LC3[5] were stored for 4 weeks at 4 °C as fully grown monolayers of $2 \cdot 10^6$ cells in 5 ml of Eagle's minimal essential medium with 10% calf serum in Müller flasks. The number of living cells was estimated in 4 flasks of each population before cold exposure and then at weekly intervals by the dye exclusion test[5]. Simultaneously with cell counting, the K^+ content of their medium was measured in individual flasks with ISMs, prepared after Walker[6] in the modification of Vyskočil and Kříž[7]. The ISMs were first calibrated in a set of standard solutions of K^+, whose composition of Na^+ was as close as possible to that of the medium, and

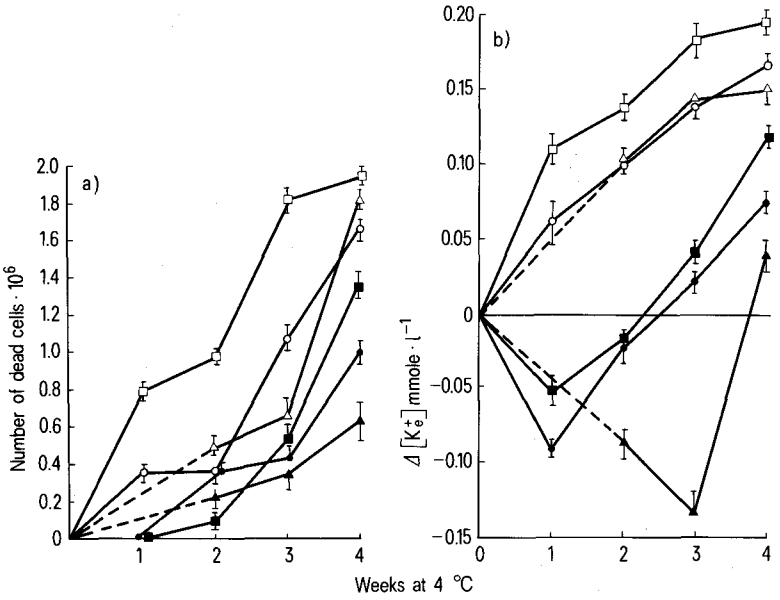

Fig. 1. *a* Mean changes in the number of dead cells ± SE in the course of 4 weeks at 4 °C in the L-As (open symbols) and the LC3 (full symbols) cells in 3 time-independent comparisons (triangles, points, squares). *b* Mean changes in medium K^+ concentration ($[K_e^+]$). Symbols as in a.

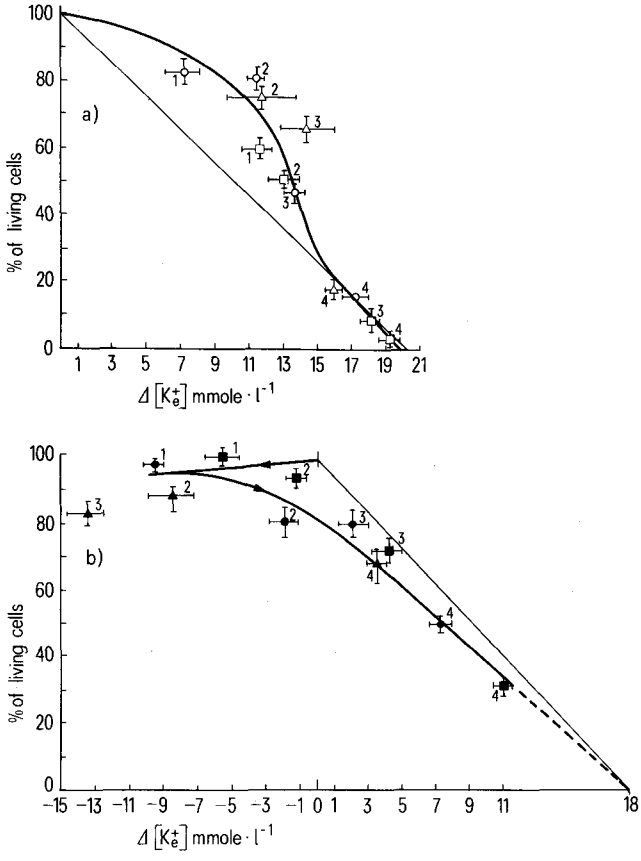

Fig. 2. *a* Expected changes in medium K^+ concentration ($[K_e^+]$) - thinner line, and actual values (triangles, points and squares, mean ± SE) - arbitrary thicker curve - for the L-AS cells. Numbers near the symbols indicate weeks at 4 °C. *b* The same for the LC3 cells.

an individual calibration curve was drawn for each electrode before and after the experiment. The results were based on 3 time-independent comparisons. For the comparison of the expected and real changes in the intracellular K^+ concentration, the content of K^+ in the cells was checked with the ISMs, by flame photometry and by atomic absorption. The medium was removed from flasks containing known numbers of cells (data obtained in sister cultures), the cells were carefully washed with 150 mmoles · l^{-1} NaCl and covered with 5 ml of 5 mmoles · l^{-1} KCl and 150 mmoles · l^{-1} NaCl, or with the same volume of deionized water. After 3 cycles of freezing to −20 °C and thawing, the K^+ content was estimated. The cell diameter of the 2 populations was measured with the electronic particle counter Celloscope 202[5], and the intracellular K^+ concentration was calculated following the formula

$$C_i = \frac{\text{the amount of potassium released into 5 ml}}{\text{number of cells} \cdot \text{mean cell volume}}$$

It was consistently higher in the L-As (220 mmoles · l^{-1}) than in the LC3 cells (160 mmoles · l^{-1}).

Results. Figure 1, a demonstrates the increase in the number of dead cells during 4 weeks in the cold, figure 1, b shows corresponding changes of K^+ concentration in the medium. The heavily damaged L-As population lost K^+ throughout the experiment, while the LC3 cells took K^+ up from medium during the 1st 2 weeks at 4 °C, and an unequivocal loss did not occur until after 4 weeks in the cold. Figure 2 shows the relation between the expected values calculated under the presumption that dead cells enriched the medium by all their K^+, and the values actually found. The actual changes agreed with those expected only in the L-As cells in the 2nd half of cold storage, while the K^+ concentration in the medium of the 1st half was higher than expected. The K^+ concentration in the medium of the LC3 cells was lower than was to be expected during the whole cold stress period.

Discussion. Comparative studies of potassium regulation in mammalian tissues and cells characterized by different degrees of cold sensitivity showed that superior survival was coupled with better regulation of K^+ concentration in the cells. Previous findings that K^+ losses estimated directly by flame photometry in cold-stored human, hamster and mouse cells preceded cell death[4,8] were confirmed by indirect measurement of K^+ changes in the medium with ISMs. This method revealed a K^+ uptake by the LC3 cells in the cold which has not previously been described, and which could not be explained by an increase in cell volume (2879 μm^3 at 36 °C, 2711 μm^3 after 1 week

and 2572 μm^3 after 2 weeks at 4 °C). Surviving cells enlarged after 3 weeks at 4 °C (3473 μm^3), but here the situation was still more complicated because of K^+ losses from cells damaged and dead at this time. Single cell data are necessary to clarify these points.

As the LC3 cells spend the majority of their life at 4 °C, changes in the properties of membrane lipids and fluidity may influence the activity of Na^+K^+-ATPase and K^+ transport in the cold. In view of the importance of K^+ for protein synthesis and mitosis[1,2,9-11], the maintenance of an adequate K^+ concentration may play an important role in the quick regrowth of the LC3 cells rewarmed to 36 °C[2].

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The effect of lidocaine on the secretion induced by cholera toxin in the cat small intestine[1]

J. Cassuto, M. Jodal, R. Tuttle[2] and O. Lundgren

Department of Physiology, University of Göteborg, S-40033 Göteborg (Sweden), 8 February 1979

Summary. The intraluminal administration of lidocaine, a local anaesthetic agent, inhibits the net loss of fluid into the intestinal lumen produced by cholera toxin in the cat. It is suggested that the activation of a nervous reflex is involved in the pathogenesis of cholera.

The profuse fluid loss in cholera is, according to current concepts, the result of a direct cellular action of the cholera toxin on the intestinal epithelium[3]. Cyclic AMP is believed to be involved in this process since the concentration of this compound in the enterocytes and in most other cells is increased after cholera toxin exposure[4,5]. It has been reported that the intestinal contents in cholera contain vasoactive intestinal polypeptide (VIP) at high concentrations[6]. Since VIP has been demonstrated to be localized only in nervous tissue in the gut[7], it was considered of interest to investigate to what extent a cholera induced secretion could be decreased by a local anaesthetic agent, indicating a possible nervous reflex involvement in the pathogenesis of cholera.

Methods. The experiments were performed on cats deprived of food for 24 h with free access to water. The animals were anaesthetized with chloralose (50 mg/kg b.wt). Venous outflow from a denervated segment of the jejunum weighing 10–15 g was recorded by an optical drop recorder unit operating an ordinate writer. Arterial blood pressure was recorded from the left femoral artery by a pressure transducer. Net intestinal transport of fluid was measured continuously with the method described in detail by Jodal et al.[8]. This technique implied the perfusion of the intestinal lumen with an isotonic electrolyte solution at a constant rate of 1 ml/min in a recirculating system. Changes in net fluid transport were registered by a volume transducer connected to the recirculating system via a T-tube. All recordings were made on a Grass polygraph.

The solution used to perfuse the lumen of the small intestine contained (mmole/l): NaCl 122; KCl 3.5; $NaHCO_3$ 25; KH_2PO_4 1.2; $MgCl_2 \cdot 6\,H_2O$ 1.2; $CaCl_2$ 2.5; mannitol 30. The osmolarity of the solution was 310–315 mOsm/kg H_2O. Lidocaine (Xylocain®; generously supplied by Astra AB, Södertälje, Sweden) was added in amounts of 1 or 2 g/l electrolyte solution. The lidocaine solution was perfused through the intestinal segment for 90 min.

About 400 mg of a crude cholera toxin (kindly supplied by Dr Jan Holmgren, Department of Medical Microbiology, University of Göteborg) was dissolved in 5–10 ml physiological saline and exposed to the intestinal segment for 30 min. The toxin was washed away with 50–100 ml warm saline.

Results. The results of 6 technically successful cat experiments are summarized in the table. It is evident that exposing small intestinal segments to cholera toxin induced a net secretion of fluid into the intestinal lumen. This occurred within 60–150 min. Changing the intestinal perfusion solution to one containing lidocaine inhibited net fluid loss into the lumen in all experiments. In 3 experiments it even produced a net fluid absorption from the lumen. It was possible to follow the net fluid transport after lidocaine exposure in 5 experiments. In all but one a net fluid loss into the lumen was again recorded within 1 h after terminating the lidocaine perfusion.

In control experiments it was shown that lidocaine itself in the concentrations used in this study did not affect net water uptake from a normal intestinal segment.

Arterial blood pressure, intestinal blood flow and intestinal fluid transport during control conditions and after cholera toxin exposure: before, during and after perfusing the intestinal segment with a lidocaine solution. – denotes a net fluid transport from tissue to lumen. Mean ± SE

	Control	Cholera		
		Before lidocaine	During lidocaine	After lidocaine
Arterial blood pressure mm Hg	113 ± 7	98 ± 8	86 ± 7	83 ± 5
Intestinal blood flow ml × min^{-1} × 100 g^{-1}	29 ± 5	34 ± 6	40 ± 5	46 ± 5
Intestinal fluid transport µl × min^{-1} × 100 cm^2 serosal $surface^{-1}$	242 ± 62	− 149 ± 40	61 ± 41	− 49 ± 32
Number of observations	6	6	6	5