

Fig. 3. Comparison of the hydrosmotic effects of norepinephrine (NE, $6 \times 10^{-8} M$) on 2 pieces of the same toad skin, in the absence, and in the presence, of La^{3+} ($LaCl_3$, $5 mM$). Technique as described in Figure 2. Note the transient stimulation of water flow by La^{3+} and the sustained block of the hormone action.

¹⁰ A. W. CUTHBERT and E. PAINTER, *J. Physiol., Lond.* **199**, 593 (1968).

¹¹ P. KRISTENSEN, *Biochim. biophys. Acta* **203**, 579 (1970).

¹² G. R. BRISSON, F. MALAISSE-LAGAE and W. J. MALAISSE, *J. clin. Invest.* **57**, 232 (1972).

In view of the strong interaction between Ca^{2+} and La^{3+} observed in many biological systems³, it is reasonable to assume that lanthanides affect Ca^{2+} influx or some other critical calcium-dependent step in the hormonal stimulus-effect coupling⁴. The results observed with theophylline do not formally contradict such a hypothesis. There is increasing evidence that theophylline does more on frog skin than simply inhibit phosphodiesterase^{10,11}, and, in pancreas, an effect of theophylline on the translocation of intracellular Ca^{2+} has been postulated^{12,13}. In addition, our data are compatible with those reported by WEISS¹⁴ in frog skeletal muscle, which showed that La^{3+} did not inhibit the effects of caffeine on muscle, in particular those concerning calcium movements.

Further studies are necessary to elucidate the site and mode of action of lanthanides in amphibian epithelia. It is apparent, however, that lanthanides are useful tools for studying the mode of action of hormones and the role of calcium in such biological processes^{15,16}.

Résumé. L'addition de lanthanides au milieu baignant la surface interne (séreuse) des épithélias d'amphibien provoque une inhibition de l'effet hydrosmotique et natriférique de l'ocytocine, de la nor-épinéphrine et de l'AMP cyclique. Le rôle d'une probable interaction calcium/lanthanides est discuté.

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¹³ W. J. MALAISSE, *Nature New Biol.* **242**, 189 (1973).

¹⁴ G. B. WEISS, *J. Pharmac. exp. Ther.* **174**, 517 (1970).

¹⁵ We thank Sandoz Ltd for a generous gift of Syntocinon.

¹⁶ Supported by SNF grants Nos 3.567.71 and 3.1300.73.

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Effect of Temperature and Concentration on the Anomalous Potassium Uptake of Thermophilic Plants

Experimental studies of temperature effects on the ion uptake (IU) of plants are being carried out in several laboratories. The present general opinion is that the intensity of IU by plants increases considerably on the rise of temperature¹⁻³. Contrary to this, it was established that an anomalous initial K^+ uptake (AIPU) and an anomalous K^+ efflux occurred in the roots of certain thermophilic plants at chilling temperatures⁴. No departure from the regular IU was revealed for NH_4^+ and anions^{5,6}. The anomaly in the K^+ uptake (PU) was diminished at lower pH, while no or very small changes could be observed in the AIPU if uncouplers were employed^{7,8}. In this short paper, some properties of the AIPU of low-salt plant roots will be briefly discussed.

Material and method. Cucumber (*Cucumis sativus* cultivar Csemege fűrtös) seedlings were grown in $5 \times 10^{-4} M$ $CaSO_4$ solution under well-controlled conditions as described earlier⁶. 6-7 cm long roots of 7-day-old plants were excised and washed in distilled water for 10 min at room temperature. About 3 g of the root material was

placed in 500 ml aerated, isotopically-labelled uptake solutions kept at different temperatures between 0 and 21 °C, for given periods of time. The temperature of the absorption solution was constant within 1 °C during the incubation process. The pH of the uptake solution was adjusted to 6.4-6.5. No noticeable change in pH occurred during the experiments. For technical reasons, ⁸⁶Rb was

¹ J. F. SUTCLIFFE, *Mineral Salt Absorption in Plants* (Pergamon Press, New York 1962).

² H. E. OBERLÄNDER, *Experientia* **19**, 298 (1963).

³ H. MARSCHNER, *Fortschr. Bot.* **32**, 43 (1970).

⁴ F. ZSOLDOS, in *Potassium Research and Agricultural Production*, Reprints of the 10th Congress of the International Potash Institute (Budapest 1974), p. 167.

⁵ F. ZSOLDOS, *Acta agron. hung.* **18**, 121 (1969).

⁶ F. ZSOLDOS, *Pl. Soil* **37**, 469 (1972).

⁷ F. ZSOLDOS, in 12th Annual Report of IAEA Research Contracts, No. 134 (Vienna 1972), p. 72.

⁸ F. ZSOLDOS, *Z. Pfl.-Ernähr. Düng. Bodenkd.* **126**, 210 (1970).

chosen as label for the potassium⁹. Before the systematic investigations, comparative studies were performed on the differences in behaviour of K⁺ and Rb⁺ during uptake. Although there was a definite preference for K⁺ over Rb⁺, the K⁺ and Rb⁺ uptake processes showed parallelism concerning both the time and the temperature-dependence. Thus, the use of ⁸⁶Rb as a label for K⁺ proved permissible for comparative studies.

The achievement of saturation in PU was dependent upon both concentration and temperature (e.g. for 10⁻⁵ M K (⁸⁶Rb)Cl solution see Figure 1). Considering

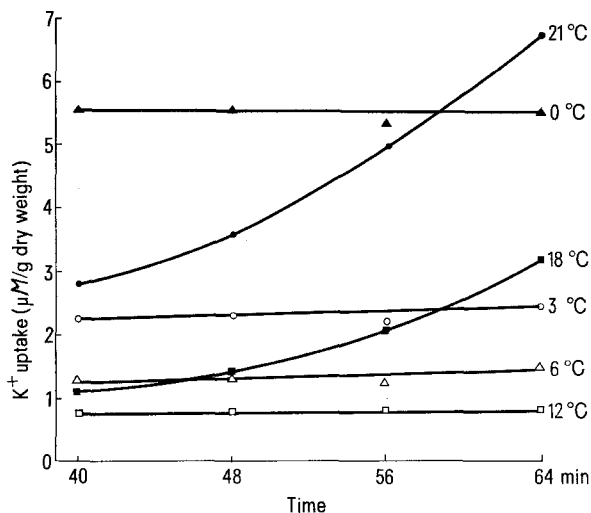


Fig. 1. The time-dependence of K⁺ uptake by cucumber roots from 10⁻⁵ M KCl solution at different temperatures.

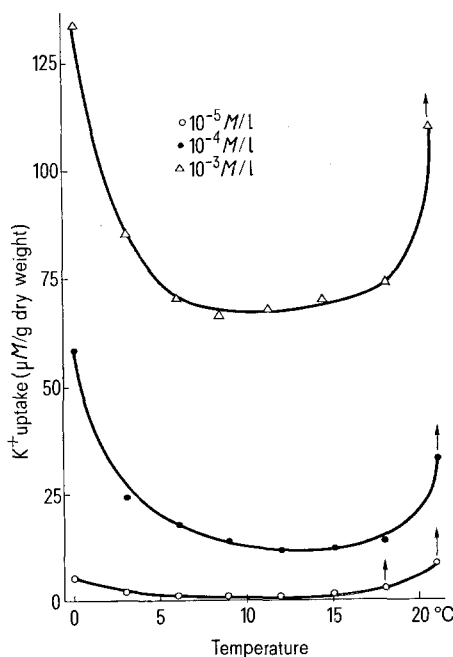


Fig. 2. The K⁺ uptake by cucumber roots at different temperatures and different K-ion concentrations of the absorption solution. Uptake time 64 min. The arrows indicate that the data are not stationary values as shown in Figure 1.

that at higher temperatures the saturation took a long time, it was practical to reduce the previously-used time of incubation to 64 min in order to avoid any irreversible physiological change of the roots. The uptake studies were carried out in 10⁻³ M, 10⁻⁴ M and 10⁻⁵ M K (⁸⁶Rb)Cl absorption solutions, as previously described⁶.

Results and discussion. The amounts of K⁺ absorbed at different temperatures are given in Figure 2, for the 3 different concentrations of the absorption solution. In all 3 cases the temperature-dependence of the PU shows a definite minimum; the higher the concentration of the absorption solution the more pronounced the minimum if the same scale is used. To eliminate the distortion of the scale, the uptake curves of Figure 2 are normalized to unity at the minimum and replotted in Figure 3. On the basis of Figure 3, the following regularities can be observed:

1. The PU exhibit a minimum between 9 and 12°C/ t_{min} .
2. The normalized minimum is the more pronounced the lower the concentration of the absorption solution.
3. There are 3 well-distinguishable ranges in Figure 3, which are separated by the demarcation temperatures t_1 and t_2 ($t_1 < t_{min}$ and $t_2 > t_{min}$ are defined as the temperatures at which the relative PU of the roots exceeds the uptake at the normalized minimum by 10%).

The PU has a negative temperature coefficient if $t < t_1$ and a positive one if $t > t_2$, and it is insensitive to temperature between t_1 and t_2 . The demarcation temperatures, and also the ranges insensitive to temperature, are concentration-dependent, as shown in the Table. These results suggest that in the roots of thermophilic plants at least 2 independent uptake systems, different in nature, operate. The process with negative temperature coefficient is certainly a passive one and probably associated with the liquid crystal-solid gel phase-transitions of membrane lipids¹⁰. This is supported by the fact that the AIPU is not, or only slightly affected by either 2,4-DNP or KCN¹¹. Since there exists a considerable osmotic gradient between roots and absorption solutions, a water + solute flow into the roots can be assumed, which may be responsible in part for the transport of K-ions. According to HOUSE and FINDLAY¹², the PU related to the water transport into the roots can be described by the expression

$$J_{K^+} = L_p \cdot R \cdot T \cdot C_s (C_r - C_s) + \Phi_w \cdot C_s \quad (1)$$

Here L_p is the hydraulic conductivity, R the gas constant, C_s the concentration of the absorption solution, C_r the concentration of free K-ions in the root, and Φ_w a water flow not of osmotic origin. It can be seen immediately that the concentration dependence of the isothermal PU follows Eq. (1) qualitatively below t_1 . Since L_p and Φ_w are to a first approximation independent of temperature¹³, there are 2 reasonable possibilities to explain the appearance of AIPU below t_1 : 1. The phase-transition of membrane lipids leads to an increasing number of statistically distributed pores, which results in enhanced electrolyte transport into the roots. 2. The driving force ($C_r - C_s$) has a negative temperature coefficient, and this causes the anomalous temperature-dependence of PU.

⁹ M. FRIED and H. BROESHART, *The Soil-Plant System in Relation to Inorganic nutrition* (Academic Press, New York, London 1969).

¹⁰ S. J. SINGER, in *Structure and Function of Biological Membranes* (Ed. L. I. ROTHFIELD; Academic Press, New York 1971), p. 145.

¹¹ F. ZSOLDOS, F. SIROKMÁN and E. CSEH, *Z. Pflanzenphysiol.* **60**, 169 (1969).

¹² C. R. HOUSE and N. FINDLAY, *J. Exp. Bot.* **17**, 344 (1966).

¹³ W. P. ANDERSON and E. J. REILLY, *J. exp. Bot.* **19**, 648 (1968).

It seems plausible to suppose that both the above mechanisms operate. As regards to the possible role of phase-transition, the concentration dependence of t_1 can be considered. It has recently been shown by TRÄUBLE and EIBL¹⁴ that the ordered-fluid phase-transition temperature of phospholipids can be lowered to a large extent by increase of either the ionic strength or the pH. Applying TRÄUBLE and EIBL's theoretical arguments to the liquid crystal-gel transition of membrane lipids, the variation of t_1 with concentration can readily be explained.

The concentration-dependence of the demarcation temperatures t_1 and t_2 , as well as that of temperature-insensitive ranges Δt ($\Delta t = t_2 - t_1$)

Concentration	$10^{-3} M$	$10^{-4} M$	$10^{-5} M$
t_1 (°C)	4.5	9.5	9.5
t_2 (°C)	17.0	17.0	14.0
Δt (°C)	12.5	7.5	4.5

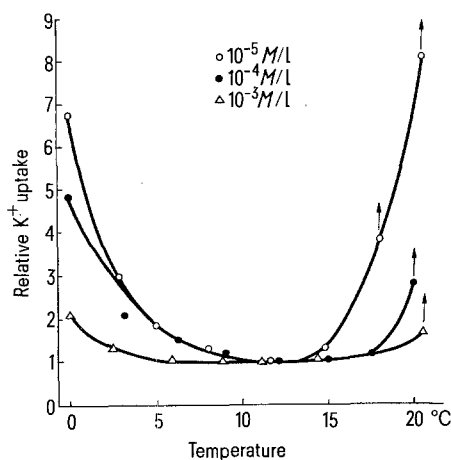


Fig. 3. Relative K^+ uptake by cucumber roots at different temperatures and different K -ion concentrations of the absorption solution. Uptake time 64 min. The arrows indicate that the data are not stationary values as shown in Figure 1.

As far as the PU above t_2 is concerned, we have no unequivocal and convincing interpretation. It is possible that a K^+ - Na^+ -activated ATPase system may be operating in the roots. The probability of such a system was apparently supported by the concentration-dependence of the demarcation temperature t_2 . Namely, if such an uptake system were involved in the PU above t_2 , the inhibitory effect of increasing K^+ concentration in the absence of Na -ions could explain the concentration-dependence of t_2 . However, no increase in the PU could be induced by adding different amounts of $NaCl$ to the absorption solution. Although there is no doubt that an active system is operating above t_2 , the basic character of this process is still completely obscure. In this connection we refer to the excellent publication by IPI, dealing with the biochemical and physiological aspects of PU by living organisms¹⁵. Similarly, no satisfactory explanation can be furnished for the nature and character of PU between t_1 and t_2 .

It seems that the mechanism of the AIPU of thermophilic plants is rather complicated and somewhat different from that of other plants. However, the understanding of these processes is of great theoretical and practical (agricultural) importance and worthy of further investigations. These are now in progress.

Zusammenfassung. Nachweis, dass in der thermophilen Gurke (*Cucumis sativus*) ein mehrphasiges K^+ -Aufnahmesystem existiert, welches sich von jenem kalteunempfindlicher Pflanzen unterscheidet. Untersucht wurden abgetrennte Gurkenwurzeln im Temperaturbereich von 0 und 21°C.

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¹⁴ H. TRÄUBLE and H. EIBL, Proc. natn. Acad. Sci. 71, 214 (1974).

¹⁵ Potassium in Biochemistry and Physiology (Publ. International Potash Institute, Berne 1971).

¹⁶ Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences, P.O.B. 521, H-6701 Szeged, Hungary.

Répercussions précoces de l'hypothyroïdie sur la synaptogénèse dans le cortex cérébelleux du Rat nouveau-né

L'hypothyroïdie entraîne, chez le jeune Rat, un ralentissement de l'augmentation de la densité des synapses dans la couche moléculaire du cortex cérébelleux. Selon NICHOLSON et ALTMAN¹, la différence ne devient significative qu'après l'âge de 15 jours, mais dans un travail précédent² nous l'avons trouvée déjà importante et hautement significative à l'âge de 14 jours. Par ailleurs les répercussions les plus nettes de l'hypothyroïdie sur la maturation histologique du cortex cérébelleux s'observent pendant la deuxième semaine post-natale³⁻⁵, et, pendant la première semaine, on note déjà des effets de la déficience thyroïdienne sur la maturation biochimique de l'organe^{3, 6-8}. De plus, un ralentissement de l'augmentation de la fraction synaptosomique du cervelet, estimée d'après son contenu en protéines, a été mis en évidence dès

l'âge de 10 jours chez l'animal hypothyroïdien⁹. L'ensemble de ces données et plus particulièrement la dernière, joint

¹ J. L. NICHOLSON et J. ALTMAN, Science 176, 530 (1972).

² A. REBIERE et J. LEGRAND, C.R. Acad. Sci., Paris 276, 2317 (1972).

³ J. CLOS, F. CREPEL, C. LEGRAND, J. LEGRAND, A. RABIE et E. VIGOUROUX, Gen. comp. Endocr. 23, 178 (1974).

⁴ J. LEGRAND, Archs. Anat. microsc. Morph. exp. 56, 205 (1967).

⁵ A. REBIERE et J. LEGRAND, Archs. Anat. microsc. Morph. exp. 67, 105 (1972).

⁶ J. DAINAT, A. REBIERE et J. LEGRAND, J. Neurochem. 17, 581 (1970).

⁷ J. GOURDON, J. CLOS, C. COSTE, J. DAINAT et J. LEGRAND, J. Neurochem. 21, 861 (1973).

⁸ J. CLOS, C.R. Acad. Sci., Paris 275, 2917 (1972).

⁹ A. RABIE et J. LEGRAND, Brain Res. 67, 267 (1973).