Potassium Uptake by *Staphylococcus aureus* and the Relation to its Water Content

Staphylococcus aureus is a bacterium with a high content of potassium[1,2], and among the non-halophilic bacteria it has the greatest resistance to high saline concentration[2]. It was chosen in this work for a study of the relations between the potassium uptake and the variations of the cellular water content. There is only fragmentary knowledge in this field. KOTYK and KLEINZELLER[3] studied, however, the modifications of cell volume in relation to the variations of the sodium content, and KLEINZELLER[4,5] and AEBI[6] studied the behaviour of sodium, potassium and water in relation to the swelling of the renal cells at 0°C and 37°C.

Material and method. Strain 22 ISI of *Staphylococcus aureus* was examined. Cells in the log-phase, centrifuged at +4°C, washed 3 times with distilled water, were divided into 4 equal samples and suspended variously in distilled water, in potassium citrate (final concentration 0.2*M*), in saccharose (final concentration 0.2*M*), and in saccharose and potassium citrate (final concentration 0.2*M*). After incubation for 30 min at 37°C in a water bath with shaking, the wet weight, dry weight and potassium content of the cells was measured in the four suspensions[1].

Results and discussion. The analysis of the means of the data of Table I, in which are given the results of 10 experiments, shows that the difference between means of water content in cells with different potassium content is significant at a level of 0.1%, whereas the difference between means of water content of the cells suspended in media with different osmotic pressure is significant at 5%. It appears also that the two experimental factors (content in cellular potassium and external osmotic pressure) act separately and that there is no interaction between them. The structure of the average response obtained is reported in Table II, particularly so as to clarify the effect made

Table II

	A_0 B_0	A_0 B_1	A_1 B_0	A_1 B_1
d = average response in absence of experimental factors *A* and *B*	67.6	67.6	67.6	67.6
a = deviation by effect of factor *A*	0.87	0.87	− 0.87	− 0.87
b = deviation by effect of factor *B*	2.2	− 2.2	2.2	− 2.2
ab	0.12	− 0.12	− 0.12	0.12
$\{AB\}$	70.8	66.1	68.8	64.6

$4d = \Sigma_{ij}\{A_iB_j\}$
$4a = \Sigma_j\{A_0B_j\} - \Sigma_j\{A_1B_j\}$
$4b = \Sigma_i\{A_iB_0\} - \Sigma_i\{A_iB_1\}$
$4ab = \Sigma_{ij}\{A_iB_j\} - \Sigma_{ij}\{A_iB_j\}$

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Table I

	A_0 Cell suspension in the absence of saccharose				A_1 Cell suspension in the presence of 0.2*M* saccharose			
	B_0 Cell suspension in the absence of potassium citrate		B_1 Cell suspension in the presence of 0.2*M* potassium citrate		B_0 Cell suspension in the absence of potassium citrate		B_1 Cell suspension in the presence of 0.2*M* potassium citrate	
	% H_2O	K^+ in m-equiv./g dry wt. of cells	% H_2O	K^+ in m-equiv./g dry wt. of cells	% H_2O	K^+ in m-equiv./g dry wt. of cells	% H_2O	K^+ in m-equiv./g dry wt. of cells
	71	(0.38)	65	(1.23)	67	(0.37)	64	(1.24)
	71	(0.42)	66.6	(1.19)	67.8	(0.43)	63.2	(1.18)
	71	(0.40)	67.3	(1.12)	70	(0.41)	65	(1.10)
	70	(0.47)	67	(1.17)	70	(0.45)	67	(1.17)
	71	(0.42)	67.6	(1.09)	71	(0.41)	66	(1.00)
	70	(0.41)	65	(1.18)	67.2	(0.41)	64	(1.10)
	71	(0.43)	67.2	(1.21)	70	(0.43)	65.3	(1.19)
	71	(0.39)	67	(1.12)	69.2	(0.38)	64.7	(1.08)
	71	(0.45)	66.5	(1.17)	67.8	(0.46)	64.2	(1.16)
	71	(0.47)	64.0 *x*	(1.20)	68	(0.47)	64.2	(1.20)
Means *x*	70.8	(0.42)	66.1	(1.17)	68.8	(0.42)	64.6	(1.14)
Deviations SS	1.60		13.76		17.76		13.06	
D.f.	9		9		9		9	

$A_0 - A_1 = 2.485$ +. $B_0 - B_1 = 6.31$ + + +. $(A_0 - A_1)(B_0 - B_1) = 0.355$ −. Student's t tabulated for 40° of freedom; $t_{5\%} = 2.021$; $t_{1\%} = 2.704$; $t_{0.1\%} = 3.551$.

by the two experimental factors on the total water content of the cells. The results show that the deviation B due to the presence or absence of potassium is greater than the deviation A due to the presence or absence of the $0.2\,M$ saccharose. From the results obtained it may be concluded that the potassium uptake by cells of *Staphylococcus aureus* is accompanied by a decrease of intracellular water. The explanation of this is related to transport mechanism and consequent on the chemico-physical state of the potassium of the cells. The concept of an active carrier transport of the potassium[7,8] is necessarily linked to that of free potassium in the cells, and in concentrations greater than external ones[9]. If the intracellular potassium were in the free state, in the tests carried out there would have been an increase in water in conjunction with the stated increase in the intracellular potassium; on the other hand, there would not have been variations in water content if the potassium were exchanged with other intracellular substances. The average quantity of potassium in excess which enters into the cells in comparison with the controls is about 0.75 m-equiv./g of dry cells; this amount, considering a water content in the cells in a free state, corresponds to a solution of about $1\,M$, which in our experimental conditions would cause an osmotic pressure of about 25 atmospheres. The external osmotic pressure, however, in the presence of only $0.2\,M$ potassium citrate or added to $0.2\,M$ saccharose, is about 5 and 10 atmospheres respectively.

All this is without calculating the other intracellular osmotically active solutes or substances. Even admitting that only 30% of the potassium is in the free state[10], the external osmotic pressure does not exceed the internal one. It is therefore logical to conclude that the potassium is not in a free state but adsorbed by means of bonds of various kinds[11] on dissociated groups of the polypeptide chains of the protoplasmatic gel, the hydration of which is modified. Groups of the polypeptide chain with anionic dissociation, in fact, are hydrated groups which repulse each other. When potassium is adsorbed, which balances the negative charge, the repulsion ceases, the distance between the polypeptide chains is reduced, and the quantity of free water decreased. Such an explanation is in agreement with the modern theories which relate the phenomena of selectivity and permeability to all cell protoplasm, the polyelectrolytic behaviour which is subject to ever more investigations[10,12,13].

Riassunto. Cellule di Stafilococco aureo in fase di crescita logaritmica incorporano potassio eliminando acqua. Lo spostamento dell'acqua intracellulare dipende più dalla incorporazione del potassio che dalla pressione osmotica del liquido di sospensione.

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The Effect of Serotonine and 5-Hydroxytryptophan on the Erythrophores of Crustaceans

The colour pattern of the prawns *Palaemon serratus* (Pennant) and *Palaemon elegans* Rathke is composed of various types of chromatophores. Of these, two kinds of red pigmented cells, the large and small erythrophores, are the most numerous. Both species of prawns possess characteristic dark bands on the cephalothorax and abdomen. These consist purely of large erythrophores, while small erythrophores are scattered between these bands.

Eyestalk extirpation of the prawns results in pigment dispersion, while injection of extracts from the eyestalk or from the post--commissure organs (PCO) induces pigment concentration in both types of erythrophores of eyestalkless specimens of either species. In a study of the colour changes of *Palaemon adspersus* Rathke, ÖSTLUND and FÄNGE[1] noticed that 5-hydroxytryptamine (serotonine) induces pigment dispersion in the erythrophores of this prawn. AOTO[2] found that this substance causes pigment dispersion in the large erythrophores of the fresh water prawn *Palaemon paucidens* De Haan. To investigate whether or not serotonine also affects the colour change of other *Palaemon* species, the erythrophore-stimulating potency of serotonine was tested in *Palaemon serratus* and *Palaemon elegans.* Injection of serotonine in doses of 1 µg or higher into intact white-background-adapted *Palaemon* results in a distinct pigment dispersing reaction of the large erythrophores only. This selective action of serotonine is also visible following injection of a mixture of PCO extract and serotonine into eyestalkless prawns. If, for instance, a mixture containing 1/200 part of one pair of PCO and 1 µg serotonine is given to eyestalkless *Palaemon serratus* or *Palaemon elegans,* a strong pigment concentrating reaction of the small erythrophores sets in immediately following injection, while such changes do not occur in the large erythrophores of these two species.

The presented results are in agreement with those of ÖSTLUND and FÄNGE[1] and AOTO[2]. These data and the fact that the presence of serotonine in crustaceans has been demonstrated by WELSH and MOORHEAD[3] indicate that serotonine may play an important role in the colour change reactions of prawns.

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