curs in the activity of Na,K–adenosine triphosphatase (*12*) and other enzymes (*13*), in the fluorescence of excitable nerves (*14*), and in calorimetric studies of lipids and water (*15*). In a few cases the thermal behavior is actually known to reflect the underlying cooperative conformational alterations.

The approach examined in the present report outlines a theoretical model that predicts the behavior of smooth muscle around the transition temperature. The model makes a quantitative prediction relating the cell electrolyte levels to temperature. A second prediction made by this model is that the transition temperature should be lowered by raising K_{ex}. Both of these predictions are quantitatively confirmed.

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2. The composition of the basic incubating medium is: NaCl, 121 m*M*; KCl, 5 m*M*; $NaHCO_3$, 22.5 m*M*; PO_4H_2Na, 1.2 m*M*; $MgCl_2$, 1.2 m*M*; $CaCl_2$, 2.5 m*M*; and glucose, 5.6 m*M*. The solution is gassed with a mixture of 95 percent O_2 and 5 percent CO_2.
3. The total potassium and sodium contents of taenia coli incubated at 36°C for periods of 13, 15, and 17 hours were (in μmole/g): 77.9 ± 2.9, 77.6 ± 2.3, and 79.7 ± 2.7 for potassium and 84.4 ± 3.1, 82.2 ± 3.4, and 84.0 ± 4.4 for sodium. The total potassium and sodium contents for another group of muscles incubated at 1.0°C for the same period of incubation were: 7.6 ± 1.5, 6.9 ± 0.7, and 7.8 ± 1.3 for potassium and 152.7 ± 2.0, 156.6 ± 4.7, and 156.5 ± 5.6 for sodium, respectively.
4. E. E. Daniel and K. Robinson [*Can. J. Physiol. Pharmacol.* **49**, 240 (1971)] have reported that the sodium efflux from rat uteri is much more dependent on temperature in the range 15° to 5°C than in the range 37° to 15°C.
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17. Sorbitol-^{14}C spaces determined at different temperatures gave a wet weight value of 0.430 ± 0.009 ml/g (30 data), which is in agreement with that reported by P. J. Goodford [in *Handbook of Physiology* (Williams & Wilkins, Baltimore, 1968), vol. 4, p. 1743].
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Cooperative Thermal Effects on the Accumulation of Potassium and Sodium in Frog Muscle

Abstract. *Sodium-rich frog muscles are found to extrude sodium and reaccumulate potassium at 0°C. The uptake of potassium by these muscles is studied at three different temperatures as a function of external potassium concentration, K_{ex}. The steady-state potassium content of the tissue is related to K_{ex} by a sigmoidal cooperative curve at all temperatures. These results are compared with findings on a mammalian smooth muscle.*

Unlike many tissues when they are cooled, frog muscle is known to retain the bulk of its potassium both in vivo and in vitro (*1*). This is seen in Table 1, where the electrolyte contents are given for muscles stored at 0°C in normal Ringer solution (*2*). In 5 days the tissue electrolytes changed less than 10 percent compared with the fresh tissue values. In contrast, many mammalian smooth muscles lose more than 90 percent of cellular potassium during overnight incubation at 0°C (*3*). We now describe the electrolyte-accumulating properties of frog muscles which, as a result of preliminary treatment, contained high sodium and low potassium concentrations. The effects of temperature on these properties are compared with those reported for mammalian smooth muscle (*4*).

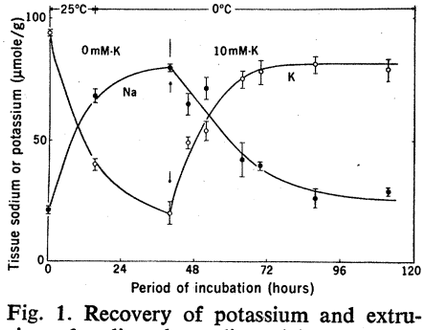

Fig. 1. Recovery of potassium and extrusion of sodium by sodium-rich muscles at 0°C. In these experiments the tissue potassium and sodium were extracted in 0.1*N* HCl overnight at room temperature. The ions were measured with a Beckman DU flame photometer.

Sodium-rich muscles in these experiments were obtained by incubating the tissues in potassium-free medium overnight at 25°C and then for 24 to 48 hours at 0°C (*5*). After this treatment, the total potassium content decreased to 10 to 30 μmole/g, and the sodium content increased to 90 to 110 μmole/g. For recovery, these muscles were transferred to a Ringer solution of known external potassium concentration, K_{ex}. The accumulation of potassium and extrusion of sodium was complete within 48 hours after the beginning of recovery at 0°C (Fig. 1) and within 20 hours at 25°C (not shown).

The ability of these tissues to reaccumulate potassium and extrude sodium against their gradients at 0°C in the presence of 10 m*M* external potassium was striking. This indicates that the potassium accumulation mechanism continues to operate at this temperature. However, potassium would be accumulated only up to 30 μmole/g when the potassium concentration of the recovery medium is 2.5 m*M*, the physiological concentration (Fig. 2A). Thus, it may be claimed on a qualitative basis that the temperature-dependent active transport mechanism is considerably slowed at 0°C. On the other hand, an alternative biophysical model was recently shown to quantitatively predict the effects of temperature on smooth muscle of the guinea pig taenia coli (*4*). The results presented below show that this model also applies to the effects of temperature on frog muscle.

The steady-state distribution of potassium and sodium is related to the external potassium concentration by a sigmoidal (S-shaped) curve (*6*). This has been considered as strong evidence that the uptake of potassium and sodium follow a cooperative mechanism (*7*). We now wish to see whether the same relationship, the cooperative adsorption isotherm, can also describe the distribution of electrolytes in the sodium-rich muscles at the end of recovery. After recovery at 25°C in solutions containing varying K_{ex} the results shown in Fig. 2A were obtained. The experimental points closely follow the theoretical curve plotted according to the cooperative adsorption isotherm by using the following parameter values (*7*): 115 ($K^{00}_{Na \rightarrow K}$), 2.7 ($n$), and 90 μmole/g (F_T). A similar set of values was used (*6*) to describe the data obtained by a somewhat different technique.

The reaccumulation of potassium by the potassium-depleted muscles was also determined at 10° and at 0°C. The results are plotted in Fig. 2, and the experimental points are in good agreement with the theoretical curves. Values of n and F_T similar to those above were used, but $K^{00}_{Na \rightarrow K}$ decreased at the lower temperatures—$K^{00}_{Na \rightarrow K}$ was 80 at 10°C and 38 at 0°C—which resulted in a progressive shift of the sigmoidal curves toward the right. A corollary of the potassium cooperative adsorption isotherm (*7*) predicts that the distribution of sodium as a function of K_{ex} in the recovery medium should follow an inverted S-curve; furthermore, the curves should be shifted to the right at lower temperatures. The experimental results at 10°C only are shown in Fig. 2B,

Table 1. Effect of incubation at 0°C on the frog muscle. The tissues were incubated in a Ringer solution containing 10 percent GIB tissue culture medium prepared according to the method described by Ling and Bohr (*6*). N, total number of tissues.

Incubation time (days)	N	Tissue K (μmole/g)	Tissue Na (μmole/g)
0*	14	89.2 ± 1.1	27.4 ± 1.3
5	12	83.0 ± 2.3	31.1 ± 1.5
10	4	72.0 ± 1.4	33.3 ± 1.2

* These control tissues were incubated in normal Ringer solution (*2*) for 1 to 3 hours at 25°C.

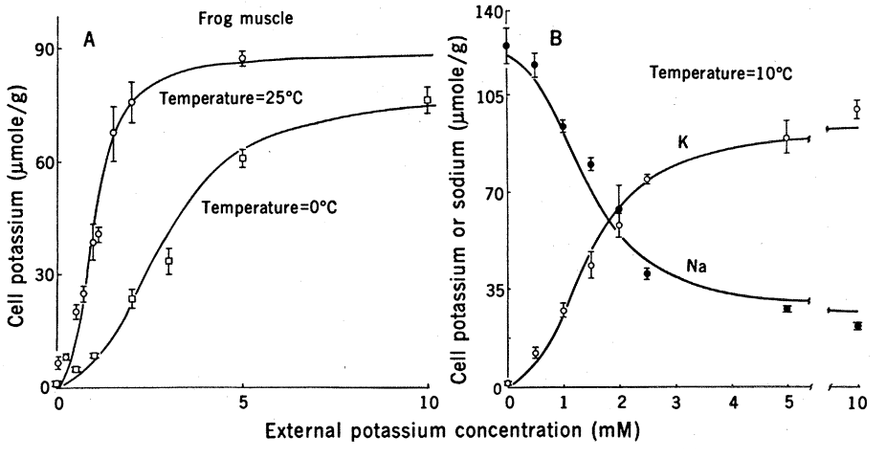

Fig. 2. Steady-state levels of cell potassium and sodium with varying K_{ex} at different temperatures. The points represent mean values of four to eight determinations. The solid lines are theoretical curves obtained from Eqs. 1 and 2 (*7*). The experiments were done by transferring the potassium-depleted muscles to a solution of known K_{ex}. They were incubated for 24 hours at 25° and 10°C. For the experiments at 0°C, the incubation period for reaccumulation was 70 hours. Cell potassium and sodium were determined from the measured values by correcting for the extracellular spaces; a value of 12.5 percent was used for the latter (*5*). This value is somewhat lower than values for the ^{14}C-inulin spaces (14 to 16 percent) which were measured on the sodium-rich muscles ($N = 8$) and which correspond to the inulin space in fresh muscles (*9*). In A the isotherms for potassium uptake are shown; the parameter values used at 25°C are given in the text. At 0°C the values were: 38 ($K^{00}_{Na \rightarrow K}$), 2.5 ($n$), and 80 μmole/g (F_T). In B the isotherms for potassium and sodium are shown at 10°C. To fit theoretical curves the parameter values used were: 80 ($K^{00}_{Na \rightarrow K}$), 2.5 ($n$), and 95 μmole/g (F_T). The theoretical curve for sodium was obtained from Eq. 2 with an additional correction of 25 micromoles of sodium per gram. This correction factor is added to Na_{ad} to account for the interstitial fraction of sodium. The data for sodium at 25° and 0°C are not shown, but they follow the theoretical curves in the same way as the data at 10°C. The cell potassium and sodium concentrations are given as wet weight.

and they are in good agreement with the theoretical predictions (*7*).

These effects of temperature are similar to those reported for a mammalian smooth muscle. In both tissues decreasing the temperature lowers the selectivity ratio $K^{00}_{Na \rightarrow K}$. However, the quantitative behavior of the two tissues is different. The tissue electrolyte contents in the smooth muscle were found to be critically dependent on the temperature. In the presence of 5 mM K_{ex} (in a physiological solution) the mammalian tissue maintained its potassium and sodium levels at temperatures between 36° and 17.5°C. A change of at least 80 percent in the tissue electrolytes occurred within the narrow temperature range of 5°C (between 17.5° and 12.5°C). The enthalpy change (ΔH^{00}) was calculated from the $K^{00}_{Na \rightarrow K}$ values and found to be about 30 kcal/mole. For the frog muscle (from data at 10° and 0°C) ΔH^{00} is only about 11 kcal/mole (*8*). The transition temperature (T_c) is lower for the frog muscle than for the mammalian smooth muscle as predicted by Eq. 3 (*8*) and the transition is not as sharp. It is possible to extrapolate a value for T_c of frog muscle from the data in Fig. 2 by plotting cell potassium against temperature with K_{ex} fixed at 2.5 mM. A value of 0° to 2°C was obtained in comparison with the value for smooth muscle of 14°C [in physiological solution (*4*)]. The high value of ΔH^{00} in the smooth muscle may be indicative of a relatively large conformational transition of the accumulating components during manipulations of the temperature.

The earlier comments regarding the stability of electrolyte contents of the frog muscle at 0°C may be reexamined. The loss of potassium at the low temperature is very slow. This slow loss of potassium, compared with the faster reaccumulation process as seen in Fig. 1, could be a result of rectification properties of the tissue which retards the outward movement of potassium from the cell (*10*). An alternative statement for this effect could be that the steady-state levels are indeed different depending on whether the net movement is outward or inward. This would be an expression of hysteresis in the cooperative accumulating mechanism.

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$$K_{ad} = \frac{F_T}{2}\left\{1 + \frac{\xi - 1}{[(\xi - 1)^2 + 4\xi n^{-2}]^{1/2}}\right\} \quad (1)$$

where K_{ad} is cellular potassium in (wet weight) micromoles per gram, F_T is the maximum level of potassium plus sodium in the cell in (wet weight) micromoles per gram, ξ is defined as $K^{00}_{Na\rightarrow K}$ (K_{ex}/Na_{ex}), $K^{00}_{Na\rightarrow K}$ is the selectivity ratio of potassium over sodium, and $n = e^{-\gamma/2RT}$, where $-\gamma/2$ is the energy of nearest-neighbor interactions. The cell sodium and potassium contents are related by

$$Na_{ad} = F_T - K_{ad} \quad (2)$$

8. It is assumed that $-RT \ln K^{00}_{Na\rightarrow K} = \Delta F^{00} = \Delta H^{00} - T\Delta S^{00}$. Here ΔF^{00}, ΔH^{00}, and ΔS^{00} are intrinsic free energy change, intrinsic enthalpy change, and intrinsic entropy change, respectively, for the exchange of sodium by potassium on a site. An expression was developed earlier (*4*) for the transition temperature (T_c) at which the cell potassium has decreased by one-half:

$$T_c = \frac{\Delta H^{00}/R}{\left(\ln \frac{K_{ex}}{Na_{ex}} + \frac{\Delta S^{00}}{R}\right)} \quad (3)$$

The behavior of taenia coli was quantitatively described by this equation at two different values of K_{ex}. Qualitatively, the behavior of frog muscle at different temperatures is also consistent with this equation.
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DDT: Inhibition of Sodium Chloride Tolerance by the Blue-Green Alga Anacystis nidulans

Abstract. Anacystis nidulans, *a freshwater blue-green alga, has been found to tolerate sodium chloride (1 percent by weight) and DDT [1,1,1-trichloro-2,2-bis-(p-chlorophenyl)ethane] (800 parts per billion) separately, but growth was inhibited in the presence of both compounds. This inhibition was reversed by an increased calcium concentration. It is possible that inhibition of (Na+,K+)-activated adenosine triphosphatase) by DDT causes this species to lose the ability to tolerate sodium chloride.*

The direct toxicities of DDT and other chlorinated hydrocarbon insecticides on algae have been studied by several research groups (*1*). They have found, however, that these chemicals are toxic to algae at relatively higher concentrations than one normally finds in nature. One important aspect of ecological studies is to discover conditions which enhance the toxicity of pollutants so as to be able to predict situations in which such chemicals can become a threat to the environment.

The role of adenosine triphosphatase in ionic transport across membranes has been well established. Recently, it has been suggested that a (Na^+,K^+)-activated, Mg^{2+}-dependent adenosine triphosphatase may be involved in the mechanism of NaCl tolerance in yeasts (*2*) and blue-green algae (*3*). Recent reports of DDT-inhibited (Na^+,K^+)-activated Mg^{2+}-dependent adenosine triphosphatase (*4–7*) and polychlorinated biphenyl inhibition of fish adenosine triphosphatase (*8*) have led us to investigate the influence of DDT on the NaCl tolerance of *Anacystis nidulans* (strain TX20), a coccoid blue-green alga obtained from the Laboratory of Algal Physiology of the University of Texas at Austin.

Algal cultures were grown in medium Cg10 (*9*) with NaCl and DDT added as needed. The growth of the test-tube cultures was followed colorimetrically according to the method of Kratz and Myers (*10*). The growth rate of the algae is expressed by the equation

$$kt = \log (N_t/N_0)$$

where k is the growth-rate constant, t is 24 hours, N_t is the cell number at time t, and N_0 is the cell number at time 0. The growth-rate value reported herein is the specific growth-rate constant, k, in logarithmic units to the base 10 per 24 hours. When $k = 0.301$, the generation time is 24 hours. The purity of the algal cultures was checked by inoculating nutrient broth (Difco) tubes with fractions of experimental algal cultures and incubating in darkness at 30°C and at room temperature. Bacterial contamination was not a problem during the course of these experiments.

The DDT-inhibition of the NaCl tolerance of *A. nidulans* was investigated by growth-rate studies. The growth-rate values in Table 1 show that *A. nidulans* was unable to grow in regular medium Cg10 + 1 percent NaCl (by weight) + DDT [800 parts per billion (ppb)]. One such culture remained colorless and optically clear after 11 days of incubation under optimum growth conditions. Three fractions of this culture were back-transferred to equal volumes of fresh media to make Cg10 + 0.5 percent NaCl + 800 ppb DDT, Cg10 + 1 percent NaCl + 400 ppb DDT, and Cg10 + 1 percent NaCl + 800 ppb DDT. The resulting growth rates of these cultures were $k = 1.24$, 1.52, and 0.0, respectively, after 3 days of incubation. Further back-transfers were made with fractions of the culture containing 1 percent NaCl + 400 ppb DDT to Cg10 and Cg10 + 1 percent NaCl. After 35 hours of incubation the growth rates of duplicate cultures in Cg10 returned to $k = 2.25$ and 2.29. Growth rates of duplicate cultures in Cg10 + 1 percent NaCl were $k = 1.58$ and 1.51. These values compared favorably with the growth rates of control cultures shown in Table 1. This result indicated that, although the growth of *A. nidulans* was suppressed at the highest combined concentrations of DDT and NaCl tested, this suppression was