

for transition III at 1 mM tris-HCl coincides with the temperature at which a dramatic increase in absorbance and a decrease in molar ellipticity occur simultaneously at 272 nm (Table I and Fig. 1). This combined with the pronounced salt dependence of the enthalpy (Fig. 2) points to a possible assignment of transition III to the DNA helix-coil transition, since it is stabilized by histone octamers in the nucleofilament.

A similar assignment for the 10 to 600 mM NaCl calorimetric profiles is compatible with the circular dichroism and absorbancy thermal profiles, which show that at ionic strengths greater than 7 mM, the helix-coil transition occurs at temperatures above 368°K. In the range 10 to 600 mM NaCl, DNA denaturation indeed cannot be monitored with standard optical techniques (6, 10), whereas DNA superhelix-helix and protein denaturation can be so monitored; their spectroscopically observed transitions occur in the temperature range of calorimetric transitions II and I, respectively.

Intact nuclei from the same rat liver cells (Fig. 1) display a highly reproducible transition (0) at a temperature close to 332 K (59°C) which is systematically absent in the isolated chromatin. In addition, the transition at 346 to 353 K (transition I), which at 10 to 600 mM yielded a constant low value of the enthalpy of transition in the isolated chromatin (see Figs. 1 and 2), dramatically increased by a factor of 4 in the intact nuclei. As indicated by circular dichroism melting curves at 223 nm, transition I appears to be related to the melting of macromolecules (such as proteins) bound to chromatin, but present in substantially increased amounts in nuclei. As expected from the equivalent NaCl normality within the sedimented nuclei, which was about 100 mM as independently evaluated by complex dielectric constant measurements (12), the melting enthalpy increased for the transition at 380 K (transition III), whereas it had a value similar to that of the 50 mM isolated chromatin for the transition at 360 to 367 K (transition II).

This is exactly what was predicted from the polyelectrolyte theory (11) for the ionic strength dependence of a quaternary chromatin structure (and stability) under the assumption that the fraction (0.30) of DNA phosphate charges neutralized by H1 histones is the same in isolated and native nuclear chromatin. The other transition (0), consistently observed at lower temperature (332 K) only in isolated nuclei, could be related to the melting of a quaternary

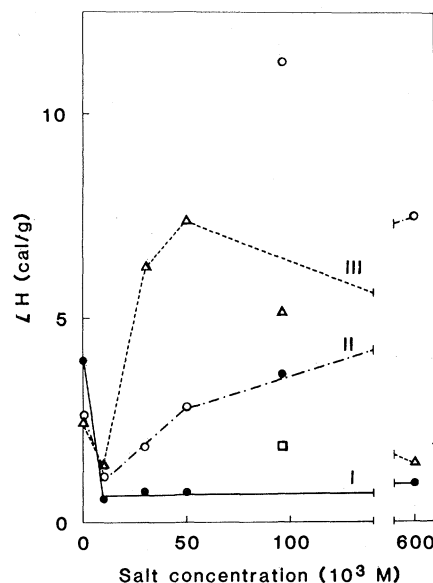


Fig. 2. The dependence of the melting enthalpy, ΔH_m , on NaCl concentration for the chromatin thermal transitions corresponding to the first (I), second (II), and third (III) peaks in the thermal profiles of Fig. 1. The values at 100 mM NaCl, equivalent to the ionic strength in native nuclei (12), is also plotted for chromatin in situ.

chromatin DNA structure (5) present only in situ.

These calorimetric data, which correlate well with viscoelastometric (13) and electron microscopic (12) data on the same nuclei, point to possibly three successive foldings of DNA in native chromatin, from tertiary (transition III) to quaternary (1-5). From pure calorimetric data, no details can be inferred about the specific geometric configuration of these higher order structures, but the number and discrete nature of these thermal transitions are compatible with only

two successive packings of nuclear DNA during interphase, behind the nucleosome—one at the quaternary (2, 3, 12) and the other at the quaternary (5, 12) level.

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Uphill Sodium Transport Driven by an Inward Calcium Gradient in Heart Muscle

Abstract. Heart cells were loaded with sodium by treatment with toxic doses of acetyl strophanthidin. After this treatment, an increase in extracellular calcium resulted in a transient net outward sodium flux against its electrochemical gradient and in net cellular uptake of calcium. It is concluded that the free energy for the net outward sodium movement was derived from the increased calcium gradient and that these ion movements took place through the sodium-calcium exchange. While in the normal physiological state the sodium-calcium exchange produces calcium extrusion from the cell, these experiments demonstrate its reversibility.

There exists, in a variety of cells, a coupled exchange of sodium for calcium (1). This exchange is thought to provide a mechanism for the outward transport of calcium that enters cardiac cells by electrodiffusion (2). Moreover, the im-

mediate energy source for this calcium transport is thought to be the large electrochemical gradient for sodium. It is conceivable that sodium-calcium exchange can reverse its direction and transport sodium out of the cell (against

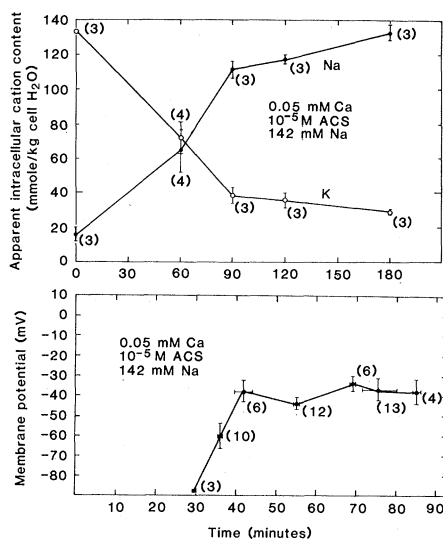
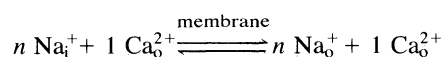


Fig. 1 (top). Effect of $10^{-5}M$ ACS on cation content in Langendorf perfused ventricular myocardium. The modified Tyrode perfusate contained 0.05 mM calcium. (●) Apparent intracellular sodium content; (○) apparent intracellular potassium content. Each point is represented by the mean $\pm$ standard error. The number of hearts contributing to each point is shown in parentheses. Fig. 2 (bottom). Effect of $10^{-5}M$ ACS on membrane potential in Langendorf hearts treated in the manner described in the legend of Fig. 1. Each point represents the mean $\pm$ standard error of the number of impalements shown in parentheses. For the first 25 minutes it was not possible to obtain stable recordings because of excessive movement of the preparation.

its electrochemical gradient) at the expense of free energy contained in the calcium gradient (3, 4). The exchange may be represented by a single hypothetical electrochemical transport reaction



where n is the stoichiometric coefficient and the subscripts i and o respectively refer to the cell interior and exterior. At equilibrium there is no net ion movement through the exchange and

$$n\Delta\bar{\mu}_{i-o}^{\text{Na}} = \Delta\bar{\mu}_{i-o}^{\text{Ca}}$$

where in each phase the electrochemical potential $\bar{\mu}^j = \mu^j + ZF\psi$ (5); μ^j is the chemical potential of species j ; Z , F , and ψ are the valence of j , the faraday, and the electric potential, respectively; and $\Delta\bar{\mu}_{i-o}$ is the electrochemical potential difference between the cell interior and exterior, that is, $\Delta\bar{\mu}_{i-o}^j = \bar{\mu}_i^j - \bar{\mu}_o^j$. With complete inhibition of the sodium pump, increasing Ca_o so that $\Delta\bar{\mu}_{i-o}^{\text{Ca}}$ exceeds $n\Delta\bar{\mu}_{i-o}^{\text{Na}}$ should produce net movement of sodium out of a cell against its own gradient and simultaneous entry of calcium down its gradient through the ex-

change. As this occurs, differences between $\Delta\bar{\mu}_{i-o}^{\text{Ca}}$ and $n\Delta\bar{\mu}_{i-o}^{\text{Na}}$ will tend to dissipate and movement of ions through the exchange will cease as a new equilibrium is approached. According to this hypothesis the driving force for ion movement through the exchange is measured by the difference $n\Delta\bar{\mu}_{i-o}^{\text{Na}} - \Delta\bar{\mu}_{i-o}^{\text{Ca}}$.

In this report we demonstrate that in heart cells inhibition of the sodium pump leads, as expected, to accumulation of sodium, which can be transiently reversed (against its electrochemical gradient) by significantly increasing external calcium. We use these observations to obtain preliminary estimates of n .

We first measured apparent intracellular sodium and potassium contents in rabbit ventricular myocardium. Rabbit hearts were rapidly excised from heparinized New Zealand White rabbits (3 to 5 kg) that had been anesthetized with sodium pentobarbital (50 mg/kg). The hearts were perfused at room temperature (20° to 23°C) in retrograde fashion through the aorta at approximately $1.5 \text{ ml g}^{-1} \text{ min}^{-1}$ with a modified Tyrode solution consisting of 118 mM NaCl, 5 mM KCl, 1 mM KCoEDTA, 1.0 mM MgCl_2 , 0.4 mM $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 24.0 mM NaHCO_3 , and 1.5 mM CaCl_2 . After 1 hour of perfusion the hearts were cut down; the ventricles were cut into three parts, dried for 24 hours at 100°C in platinum crucibles, and reweighed. From the difference in blotted and dried weight the water content was obtained. The pieces of dried ventricular tissue were ashed at 520°C for 24 hours and the ash dissolved in 0.1N HCl. The volume was made up to 10 ml and sodium, potassium, cobalt, and calcium in the samples were measured by conventional atomic absorption spectrophotometry (Perkin-Elmer model 360). Apparent extracellular water was calculated from the distribution of CoEDTA (6). Apparent intracellular sodium, potassium, and calcium content was calculated by deducting the apparent extracellular ion content from the total ion content. The ion contents of each heart were obtained by summing the contents in each tissue slice. Measurements on a particular slice were rejected if they were more than 2 standard deviations outside the mean for the whole heart. The apparent intracellular sodium content was 15.1 ± 3.4 mmole per kilogram of cell H_2O [mean $\pm$ standard error (S.E.); $N = 3$ hearts] and the potassium content was 132.5 ± 4.8 mmole/kg ($N = 3$).

We next perfused rabbit hearts that had been equilibrated for 1 hour in standard Tyrode solution with a modified

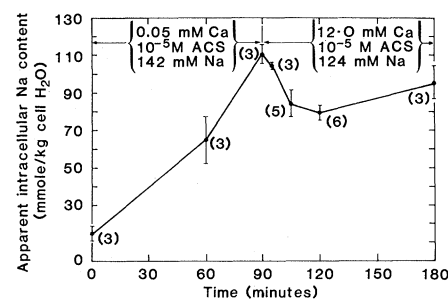


Fig. 3. Effect on sodium content of increasing external calcium from 0.05 to 12.0 mM in ACS-intoxicated ventricular myocardium. Each point represents the mean $\pm$ standard error of the number of hearts shown in parentheses.

Tyrode solution in which the calcium concentration was reduced from a nominal value of $1.5 \times 10^{-3}M$ to $5 \times 10^{-5}M$ and to which acetyl strophanthidin (ACS) had been added at a concentration of $10^{-5}M$, sufficient to block the sodium pump. Figure 1 shows the resulting time course of intracellular sodium accumulation and potassium loss. During these ion content changes the sum (sodium content plus potassium content) remains approximately constant, as does the apparent intracellular H_2O .

The time course of membrane potential change was measured with glass micropipettes filled with 3M KCl and with a resistance of 5 to 20 megohms. Multiple stable impalements were obtained from the surface of perfused ventricles during 90 minutes of exposure to $10^{-5}M$ calcium. The results of this experiment are shown in Fig. 2. There is a progressive tendency for the cells to depolarize to values that lie between -30 and -40 mV. No impalements showed potentials positive to -10 mV. Thus sodium accumulation during 90 minutes of ACS application is in the direction of dissipation of the inwardly directed electrochemical gradient for sodium.

After 90 minutes of exposure to $10^{-5}M$ ACS and $5 \times 10^{-5}M$ calcium, the external calcium concentration was increased to 12.0 mM. In order to retain osmotic balance, the sodium concentration was reduced from 142 to 124 mM. At this time the apparent intracellular sodium content was 111.2 ± 4.2 mmole/kg ($N = 3$) and the membrane potential was always negative. Since the intracellular sodium activity coefficient is unlikely to exceed the extracellular one, we may safely conclude that at the time of high calcium intervention the electrochemical sodium gradient was still inwardly directed. Within 5 minutes (Fig. 3) intracellular sodium content fell to $104.7 \pm$

1.5 mmole/kg (mean $\pm$ S.E.; $N = 3$) and within 15 minutes to 84.1 ± 7.0 mmole/kg ($N = 5$). This transient loss of sodium was highly significant ($P \leq 0.05$) with slow reaccumulation of sodium beginning 30 minutes after high calcium intervention.

Apparent intracellular calcium increased after high calcium intervention. After 5 minutes it was 6.16 ± 0.56 mmole/kg (mean $\pm$ S.E.; $N = 3$) and after 15 minutes 13.08 ± 2.2 mmole/kg ($N = 4$). This represents a significant ($P \leq 0.05$) accumulation (6.9 mmole/kg) of intracellular calcium between 5 and 15 minutes. Since extracellular calcium is unlikely to change after the first 5 minutes, the change in apparent intracellular calcium content should represent the true intracellular accumulation of calcium. During the same 10-minute period there was a sodium loss of 20.6 mmole/kg. The ratio of this sodium loss to the calcium accumulation is 3.0. Given that independent passive fluxes are not significant, sodium extrusion through sodium-calcium exchange driven by the calcium gradient would have a stoichiometric coefficient of 3.0. This is in agreement with the ratio estimated with a sarcolemmal vesicle preparation by Pitts (7). Any simultaneous inward electrodiffusive movement of both sodium and calcium must lead to underestimation of the stoichiometric coefficient for the sodium-calcium exchange; our value of 3.0 must therefore be a minimum.

The net loss of sodium can be attributed wholly to sodium-calcium exchange only if there is no transport through $\text{Na}^+ + \text{K}^+$ -stimulated adenosinetriphosphatase. After the hearts were treated for 90 minutes with $10^{-5}M$ ACS and $5 \times 10^{-5}M$ calcium, the potassium content was 38.4 ± 3.6 mmole/kg (mean $\pm$ S.E.; $N = 3$). Fifteen minutes after the high calcium intervention the intracellular potassium content was 52.2 ± 5.9 mmole/kg ($N = 6$). This accumulation of intracellular potassium was not statistically significant (Student's t -test; $P \leq 0.05$). However, a small but real gain in potassium upon high calcium intervention would, owing to the relatively few inherently scattered determinations, be expected to remain statistically insignificant. Stimulation of either sodium-potassium exchange or an electrogenic sodium-calcium exchange and attendant membrane polarization sufficient to produce an inward electrochemical gradient for K^+ would lead to net K^+ accumulation. We therefore propose that the observed potassium accumulation is real. Sodium-potassium exchange is unlikely to be the source of this accumulation

because the entire experiment was conducted in the presence of high levels of ACS. Moreover, there is no precedent for the view that calcium is capable of reactivating the sodium-potassium exchange. Increased inhibition of $\text{Na}^+ + \text{K}^+$ -adenosinetriphosphatase due to high Ca_i has been reported (8) and may be expected in this preparation owing to the measured calcium accumulation accompanying the sodium loss. Although we were not able to measure E_m during the contracture that developed in high Ca_o , we believe that the potassium accumulation resulted from membrane polarization. In the absence of sodium-potassium exchange, the most likely source of this polarization is stimulated electrogenic sodium-calcium exchange.

We conclude that the step increase in the calcium gradient at 90 minutes was the source of free energy for the observed sodium transport. Since it does not seem possible that an increase in external calcium could make the membrane potential so positive that net outward electrodiffusion of sodium would occur, we consider that the sodium extrusion is coupled to calcium influx through the sodium-calcium exchange system. Because it appears that at least three Na^+ must exchange for each Ca^{2+} , Na^+ extrusion should be accompanied by increased membrane polarization.

Since a diffusional barrier may have limited efflux rates and our observations were not made at very short intervals, we cannot calculate the initial rate of

exchange. However, the parameter $3\Delta\bar{\mu}_{\text{Na}} - \Delta\bar{\mu}_{\text{Ca}}$, which varies during an action potential, governs the direction of the sodium-calcium exchange. Thus the exchanger may make some contribution to the shape of the cardiac action potential if the stoichiometry is anything other than two Na^+ to one Ca^{2+} . Current contributions during cardiac action potentials by the exchangers, as proposed for the sodium-calcium exchange by Mullins (4) and for the Na/K exchange by Chapman *et al.* (9), should be considered.

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Membrane Isolation Alters the Gel to Liquid Crystal Transition of *Acholeplasma laidlawii* B

Abstract. *The gel to liquid crystal phase transition of membrane lipids of live Acholeplasma laidlawii B was monitored by infrared spectroscopy. It was found that, while isolated membranes are predominantly in the gel phase at the growth temperature, the live cell membranes contain a large liquid crystal phase component.*

We report here the results of a comparison between the gel to liquid crystal phase transition of plasma membranes of live *Acholeplasma laidlawii* B and that of the isolated membranes. Our results are relevant to recent studies of membrane lipids (1-7), particularly studies of lipids obtained when growth on saturated fatty acids occurs in the presence of avidin, an endogenous fatty acid synthesis inhibitor (1). The resultant fatty acid-homogeneous plasma membranes and their lipid extracts exhibit well-defined gel to liquid crystal phase transitions, and it has been

estimated that at the growth temperature only 10 to 20 percent of the lipids are in the liquid crystalline phase (4, 6). This proportion is low compared to that obtained with less homogeneous membranes (3, 7) and *Escherichia coli* (8).

However, with one exception (4) these studies have been performed on isolated membranes. It is critical to establish whether the properties of such membranes differ from those of the living organism. Recently reported ^2H nuclear magnetic resonance (NMR) spectra of intact cells were, within the signal-to-