1.5 mmole/kg (mean ± 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 ± 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 $Na^+ + K^+$–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 ± 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 $Na^+ + K^+$–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 $3\Delta\tilde{\mu}_{Na} - \Delta\tilde{\mu}_{Ca}$, 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.

JOHN H. B. BRIDGE*
JAMES B. BASSINGTHWAIGHTE
Center for Bioengineering, University of Washington, Seattle 98195

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* Present address: Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah, Salt Lake City 84132.

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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-

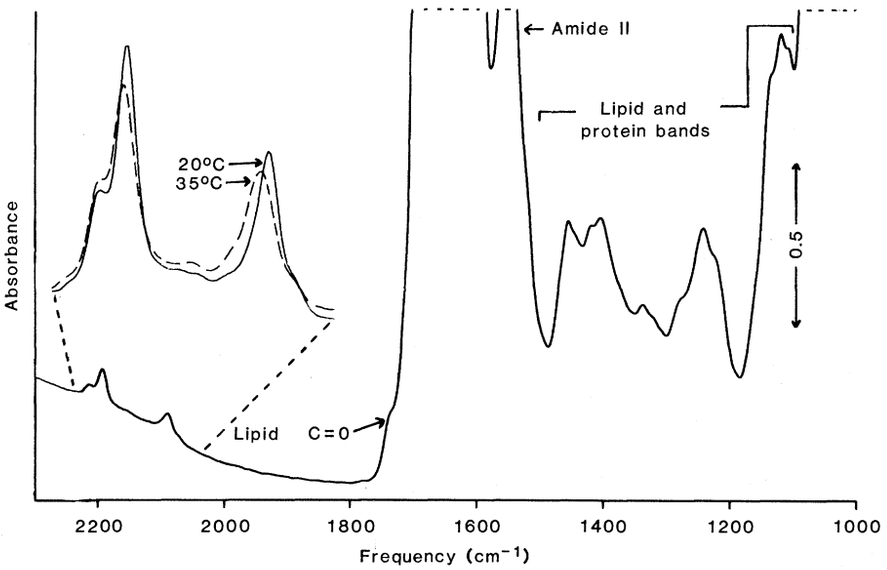

Fig. 1. Infrared spectrum of *Acholeplasma laidlawii B* grown at 30°C and recorded at 30°C. The C–D stretching region at 20°C and 35°C shows the changes resulting from transition of the membrane lipids from the gel phase (20°C) to the liquid crystal phase (35°C).

noise ratio and other constraints of the experiment, the same as those of the isolated membranes (*4*).

Acholeplasma laidlawii B were grown at 25° or 30°C in a fatty acid–depleted medium supplemented with perdeuteromyristic acid (Merck Sharp & Dohme) and avidin (25 U/liter; Sigma grade II). The cells were harvested and washed twice with 25 m*M* Hepes (*9*) containing 20 percent sucrose. About 20 mg of the suspended cells was assembled in a BaF_2 infrared cell with a 50-μm path length.

The BaF_2 cell was immediately placed in a thermostated mount in a Fourier transform infrared spectrometer (Digilab FTS-11) equipped with a highly sensitive mercury–cadmium telluride detector (Infrared Associates). Data aquisition was fully automated (*10*) so that one spectrum at 8 cm^{-1} resolution was collected every 10 seconds while the temperature was raised from 20° to 39°C and then reduced to 16°C. Measurements were restricted to this range in order to preserve the viability of the *A. laidlawii B*. Two hundred spectra were collected during 33 minutes. The bacteria were then removed from the cell, examined for lysis, and cultured in growth medium or on agar gel at 37°C. The cells were 98 to 99 percent intact, both cultures were successful, and there was no evidence of contamination. Fatty acid analysis indicated 75 and 73 percent enrichment in the myristate chain in the cultures grown at 25° and 30°C, respectively.

The plasma membranes were isolated from the remainder of the cells by standard procedures (*11*). The membranes (washed only, not lyophilized) were studied in a 50-μm BaF_2 cell over the temperature range 5° to 50°C. Longer collection times (~ 3 minutes per spectrum) were employed and 45 spectra were collected.

For each temperature and fatty acid the complete experiment was repeated three times. In all cases consistent results were obtained.

Figure 1 shows the 2300 to 2000 cm^{-1} region of the infrared spectra of live *A. laidlawii B*. The region 1800 to 1000 cm^{-1} contains a variety of bands from the lipids and proteins. However, the temperature-dependent C–D stretching bands of the fatty acid acyl chains occur near 2200 cm^{-1}, a region free from interfering bands. Since the deuterated fatty acid is incorporated as a lipid acyl chain, these bands provide a specific probe of the membrane. The frequencies of the CD_2 (and CH_2) stretching bands of acyl chains depend on the degree of conformational disorder and hence can be used to monitor the average *trans-gauche* ratio in the lipid ensemble. A frequency of 2090 cm^{-1} for the symmetric CD_2 stretching band is characteristic of a highly ordered gel phase with a low *gauche* population. Higher frequencies,

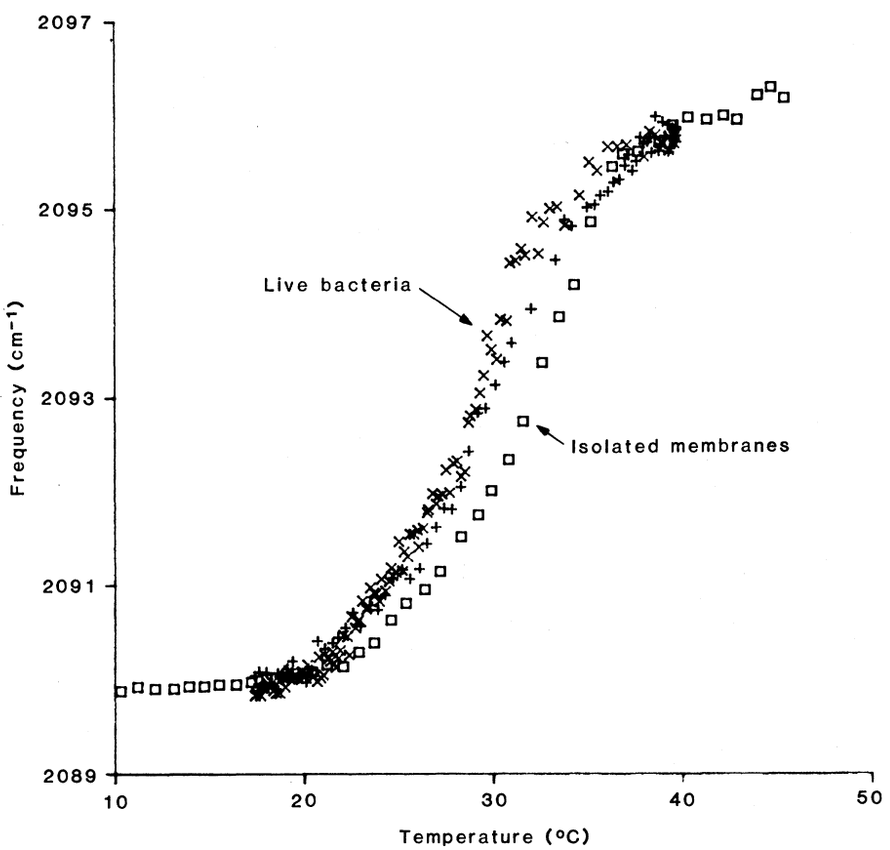

Fig. 2. Temperature dependence of the frequency of the CD_2 symmetric stretching band of the lipids of *A. laidlawii B* grown at 30°C on perdeuteromyristic acid in the presence of avidin. Shown are frequencies from spectra of live cells with the temperature ascending from 20° to 39°C (+) and descending from 39° to 16°C (×) and frequencies from spectra of isolated membranes with the temperature ascending from 5° to 45°C (□). Frequencies were determined by computing the center of gravity of the topmost 2 cm^{-1} segment of the band following interpolation to one data point per reciprocal centimeter. This method permits precise determination of small frequency shifts (*15*).

the upper limit being about 2096 cm^{-1}, are indicative of an increased population of *gauche* rotamers, that is, a transition to the liquid crystalline phase (*12*). However, the relation between frequency and conformational disorder is not linear (*13*). This necessitates the use of a simple two-component overlapping band model, analogous to that employed in ^2H NMR studies (*4*, *6*), to obtain the proportions of gel and liquid crystal phases (*14*).

Figure 2 shows temperature plotted against frequency of the CD_2 symmetric stretching band in the spectra of the live cells and the isolated membranes. Below 20°C, membranes of live cells are in the gel phase; between 20° and 34°C they undergo a transition to the liquid crystalline phase. On cooling the system exhibits slight hysteresis but reverts to the gel phase at low temperature. At the growth temperature (30°C) the frequency is 2093.3 cm^{-1}. Use of the two-component model indicates that at least 50 percent of the lipids are in the liquid crystalline phase at this temperature.

The isolated membranes also undergo a gel to liquid crystal phase transition. However, while the widths of the transitions are the same for isolated membranes and live bacteria, the transition of the former occurs at a temperature about 4°C higher. At the growth temperature the frequency of the CD_2 stretching band in the spectrum of the isolated membranes is 2092 cm^{-1}. That is, the liquid crystalline phase content is only about 20 percent, as compared to the 50 percent content of the live cell membranes. However, the general form and width of the transition and the liquid crystalline content of the isolated membranes are in excellent agreement with the results of a recent ^2H NMR study of *A. laidlawii B* grown at 30°C on perdeuteromyristic acid (*4*).

A comparison was also made between transitions of live cell and isolated membranes from a culture grown at 25°C. The reduction in growth temperature results in decreased fluidity in both systems, to the extent that the isolated membranes were effectively gel phase (frequency, 2090 cm^{-1}) at 25°C. However, the band was at 2092 cm^{-1} in the spectrum of the live cells, indicating a liquid crystalline phase content of at least 20 percent and confirming the trend observed with the culture grown at 30°C.

These data confirm that, at the growth temperature, membranes isolated from *A. laidlawii B* grown on saturated fatty acids in the presence of avidin are highly ordered, and that only a small proportion of their lipids is in the liquid crystalline phase. However, in the membranes of live *A. laidlawii B* a much higher proportion of the lipids is in the liquid crystalline phase at the growth temperature. Hence, under these growth conditions, data on conformational order obtained from isolated membranes are not always directly applicable to the live microorganism.

Since both series of spectra were recorded with the same buffer, there appear to be two potential causes for the difference between live cells and isolated membranes: (i) the process of extraction changes the intrinsic properties of the membranes and (ii) part of the mechanism for regulating membrane fluidity is extrinsic to the membranes, and hence is eliminated by membrane isolation.

DAVID G. CAMERON
ADÈLE MARTIN
HENRY H. MANTSCH
Division of Chemistry,
National Research Council of Canada,
Ottawa, Ontario, Canada K1A 0R6

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Trypsin Inhibition by Mouse Serum: Sexual Dimorphism Controlled by Testosterone

Abstract. *The trypsin inhibiting activity in the serum of male mice is substantially greater than that in females. In five strains of mice and two large groups of interstrain hybrids this difference ranged from 14 (in ICR mice) to 55 percent (in DBA mice). Castration of males significantly decreased the serum trypsin inhibiting activity, whereas the administration of testosterone restored the activity to its original level. Administration of testosterone to female mice increased the activity to a level similar to that in males of the same strain. Because almost all the change in inhibiting activity occurred in the electrophoretic α-1 region, α-1-antitrypsin is probably responsible for this effect.*

Several protease inhibitors occur in mammalian serums (*1*). One inhibitor in human serum, known as α-1-antitrypsin (or α-1-protease inhibitor), is responsible for approximately 90 percent of the trypsin inhibitory activity of the serum (*2*).

A homologous protein has been identified in mouse serum (*3*). Differences in the trypsin inhibiting activity of serums from inbred strains of mice have been observed (*4*), although the magnitude of these differences has probably been overestimated (*5*). Higher trypsin inhibiting activity in males than in females has been reported (*4*), but detailed measurements on large groups of animals have not been made. In this report, we demonstrate that the trypsin inhibiting activity in the serum of male mice is substantially higher than that in the serum of females. We also show that androgens are a major determinant for the higher trypsin inhibiting activity in males.

To measure the trypsin inhibition of many individual samples we developed a semiautomatic quantitative method (*6*) that allows measurements of 50 to 100 samples per day. All strains of mice (*7*) showed a significant male-female difference in trypsin inhibiting activity, rang-