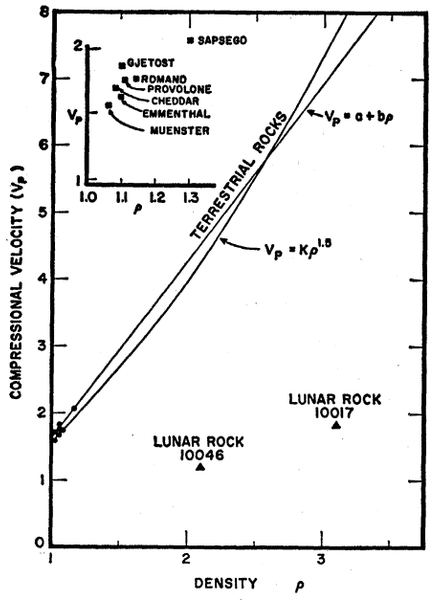

Fig. 1. Comparison of the velocity of sound for rocks with that of earth materials.

ture of the moon (*7*), and a significant group of materials was found which have velocities that cluster about those actually observed for lunar rocks.

These materials are summarized in Table 1, where, for emphasis, common rock types found on earth are listed for comparison. The materials studied were chosen so as to represent a broad geographic distribution in order to preclude any bias that might be introduced by regional sampling. It is seen that these materials exhibit compressional velocities that are in consonance with those measured for the lunar rocks—which leads us to suspect that perhaps old hypotheses are best, after all, and should not be lightly discarded.

A comparison of these low velocity materials with the predictions of Birch and of Anderson is shown in Fig. 1. It is at once apparent that these materials do yield values of velocity that are predicted by these relations for their densities. Thus the curve of Birch for the rock types diabase, gabbros, and eclogites fit the cheeses surprisingly well. This apparent inconsistency, in that the cheeses do obey these relationships by having a velocity appropriate to their density, in contrast to the lunar rocks with which they compare so well, may readily be accounted for when one considers how much better aged the lunar materials are.

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14 April 1970

Calorimetric Detection of a Membrane-Lipid Phase Transition in Living Cells

Abstract. *The membrane lipids in living* Mycoplasma laidlawii *exhibit a phase transition characteristic of that from crystal to liquid crystal within the bilayer conformation. The transition occurs at the same temperature in viable organisms, membranes isolated from the organisms, and isolated membrane lipids. The enthalpy of the transition in the membrane is compared with that of an aqueous suspension of isolated membrane lipids. The result is consistent with presence of an extended lipid bilayer in the native membrane.*

Membranes of *Mycoplasma laidlawii* undergo a reversible endothermic phase transition at a temperature dependent on the lipid composition of the membrane (*1*). That is, the more unsaturated the fatty acid chains of the membrane lipids are, the lower the transition temperature is. Aqueous dispersions of extracted lipids have transitions at the same temperature as the membranes from which they were extracted (*1*). These results were interpreted as evidence for the bilayer conformation of lipids in the membrane by analogy with the well-established behavior of aqueous dispersions of phospholipids, which exhibit crystalline to liquid crystal transitions of the hydrocarbon tails within the bulk lamellar array (*2*, *3*). This interpretation has been corroborated (*4*) with low-angle x-ray diffraction, which showed that the spacings below the transition are characteristic of a lattice of hexagonally packed hydrocarbon chains within a lamellar array. The x-ray results rule out extensive hydrophobic associations of lipid and protein.

We report here that the membranes of viable *M. laidlawii* undergo this transition and that the transition occurs at the same temperature in the organisms, membranes isolated from the organisms, and lipids extracted from the membranes. We also report the enthalpy per gram of lipid for the transition in intact membranes and in aqueous suspensions of the extracted lipids.

Mycoplasma laidlawii (strain B, PG9) were grown at 37°C in lipid-free tryptose medium supplemented with palmitic acid (*5*, *6*). Under these conditions, 65 to 70 percent of the fatty acids of the membrane lipids are palmitate. Growth was monitored by measuring optical density at 500 nm with a Zeiss spectrophotometer. Cells in the middle of the logarithmic phase of growth were collected at 19½ hours by centrifugation and washed with 0.25*M* NaCl at 4°C. Membranes were prepared by osmotic lysis (*5*) followed by centrifugation at 100,000*g* for 50 minutes at 4°C. The membranes were resuspended in a buffered medium of *p*H 8 consisting of 0.03*M* tris(hydroxymethyl)aminomethane, 0.2*M* NaCl, and 0.005*M* $MgCl_2$. They were then resedimented for calorimetry.

Lipid was extracted from the membranes by the method of Folch *et al.* (*7*). All protein remaining in the organic phase was denatured by evaporation to dryness and removed by filtration. The lipids were dried to constant weight under vacuum to remove all traces of organic solvent before they were suspended in the buffer for calorimetry. The amount of lipid per unit weight of membrane pellet was determined by weighing the lipid recovered by the extraction procedure described above.

Calorimeter scans were made in a Perkin-Elmer DSC-1B differential scanning calorimeter with a modified sample head at full-scale sensitivity of 1 mcal per second and a scan rate of 5° per minute. Scans were begun at 0°C, and stearic acid was used to calibrate the apparatus (temperature and power).

Gold-plated brass sample cells were used. Samples consisted of approximately 150 mg of a pellet of whole cells (10 to 20 percent dry weight), 150 mg of membrane pellet (10 to 15 percent dry weight and approximately 3 percent lipid), or 5 to 10 mg of membrane lipid suspended in 150 μl of buffer. Supernatant growth medium was used in the calorimeter reference cell for scans of whole organisms, and buffer served as reference for scans of membrane and extracted lipids. The samples of membrane and membrane lipid were prepared from the same batch of cells from which scans of whole organisms were made.

A control experiment was performed to determine whether the organisms observed in the calorimeter were viable. At 19½ hours a portion of a culture grown at 37°C (Fig. 1A) was collected by centrifugation and placed in a calorimeter sample cell. The temperature was lowered to 0°C and held there for 10 to 15 minutes, then raised to 37°C to simulate an actual calorimeter scan. The organisms were then resuspended into growth medium from which they were harvested. The culture was incubated again at 37°C, and growth was monitored (Fig. 1B). Growth resumes without a detectable lag. The initial optical density of the resuspended cells is slightly lower than that prior to harvest because of incomplete transfer of the organisms from the sample cell. The proportion of organisms surviving the manipulations at 19½ hours can be calculated (*8*) from Fig. 1B by comparing the slopes of logarithmic plots of growth against time for initial growth (circles) and renewed growth (crosses). The incomplete transfer of organisms from the sample cell does not affect this calculation. The calculation indicates that 80 ± 10 percent of the organisms carried through centrifugation and calorimetry up to 37°C remain viable. The error reflects the uncertainty in slope determinations from the log plots.

The lipid transition in intact cells of *M. laidlawii* is approximately 25° wide, is reversible, and begins (well below the temperature at which the cultures grew) at about 20°C (Fig. 2A). Membranes isolated from the same preparation produced the thermogram shown in Fig. 2B. The heat of transition per gram of membrane lipid, which is proportional to the areas under the peaks, is the same for whole cells and membranes. The transition temperatures are also the same. A second endotherm occurs at a higher temperature in Fig. 2, A and B. In contrast to the phase change in the membrane lipids, this higher-temperature transition is irreversible and does not appear on repetitive scans of the same sample (Fig. 2C). It occurs at approximately the same temperature even when the temperature of the lipid phase-change is drastically varied by force-feeding the organisms with various fatty acids (*1*). An extreme example is that of membranes enriched in oleate (*1*), in which the irreversible peak also occurs between 50° and 70°, and the lipid transition is at −20°C. Optical circular dichroism patterns in the 200- to 300-nm range show that an irreversible change in protein conformation accompanies the higher-temperature peak, but no appreciable change is observed over the 5° to 45°C range. Furthermore, this second calorimetric peak is greatly reduced by treating the membranes with pronase. It is reasonable, therefore, to attribute the high-temperature peak to protein denaturation. Note that the lipid and protein transitions are separate events, and that neither the area nor the shape of the lipid peak changes after protein denaturation.

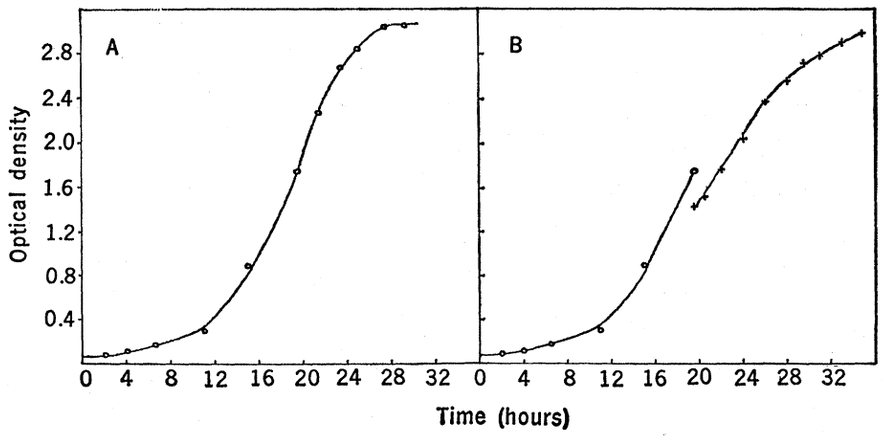

Fig. 1. Effect of centrifugation and calorimetry upon the growth of *Mycoplasma laidlawii* at 37°C, monitored by optical density at 500 nm. (A) Growth to stationary phase of undisturbed control culture; (B) growth of cells prior to calorimetry (circles), and renewed growth following the simulated calorimeter experiment described in the text (crosses).

The temperature and width of the phase change in an aqueous suspension of extracted, protein-free membrane lipids are the same as for the membranes and whole cells (Fig. 2D). The height of the peak is greater only because the sample contained more lipid than was present in the previous two scans; the endotherms of lipid and membrane can be superimposed if the size of the lipid sample is reduced.

Since 80 percent of the organisms are alive well into the lipid transition at 37°C, we conclude that transition in membranes, beginning at about 20°C, occurs in viable cells. The cells are not viable, of course, after being heated to temperatures at which denaturation of protein occurs, but the lipid phase-change occurs in a temperature range in which the cells are viable. The transitions reported for isolated membranes (*1*), therefore, do not result from a change in lipid conformation during preparation.

The heat of transition for the aqueous suspension of isolated lipids is 3.9 ± 0.2 cal per gram of lipid, where the error represents the extremes of four measurements. This value is less than

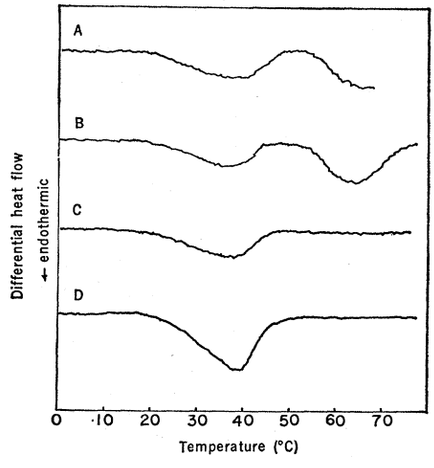

Fig. 2. (A) Differential calorimeter scan of cells of *Mycoplasma laidlawii* in the logarithmic phase in growth medium. (B) Initial scan of membranes from same preparation of cells shown in (A), suspended in buffer; (C) second scan of the same membrane preparation after denaturation of protein; (D) scan of protein-free lipids extracted from membranes and suspended in buffer.

the enthalpies reported for synthetic systems (*2*), which are about 10 cal/g. The heat of transition measured in the membrane is 3.6 ± 0.4 cal per gram of lipid where the error represents the extremes of eleven measurements of three samples.

If we assume that the structure of the aqueous suspension of lipid is an unperturbed, two-dimensional bilamellar array (*9*), the enthalpy per gram of lipid should correspond to the heat of transition for a completely extended bilayer. Any perturbation of a continuous lipid bilayer in the membrane, such as permanent aqueous pores, penetrating protein, or hydrophobic association of lipid with protein, would disrupt the cooperative interaction of fatty acid chains and decrease the heat of transition. The ratio of the transition enthalpy per gram of lipid in membranes to that for the isolated protein-free lipids is therefore an indication of the proportion of the lipids in the membranes which are in the bilayer state. If this ratio is close to 1, the bilayer must be quite extensive. The observed ratio is 0.9 ± 0.1, and suggests that 90 ± 10 percent of the lipid in the membranes is in the bilayer conformation. The calorimetric data, together with those from x-ray diffraction analyses (*4*), are thus consistent with the presence of an extensive lipid bilayer in the membrane of *Mycoplasma laidlawii* at growth temperature, and not with appreciable hydrophobic association of lipid and protein. However, the results do not eliminate the possibility that small amounts of protein might penetrate the bilayer.

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20 February 1970; revised 27 April 1970

Atmospheric Oxygen in 1967 to 1970

Abstract. *Observations of atmospheric oxygen in clean air between 50°N and 60°S, mainly over the oceans, yield an almost constant value of 20.946 percent by volume in dry air. Since 1910 changes with time over the globe appear to be either zero or smaller than the uncertainty in the measurements.*

In May 1966, the late Lloyd Berkner urged the Office of the President's Science Advisor to measure oxygen in the clean atmosphere. He justified his request in a Memorandum for the File, which was prepared jointly with L. C. Marshall and dated 29 April 1966, entitled "Potential Degradation of Oxygen in the Earth's Atmosphere." Here it was noted "that fish in the Newfoundland Banks contain significant . . . quantities of herbicides and insecticides." These, it was argued, derive from unicellular organisms, "the grass of the sea. . . . Thus, in the absence of more precise information, it must be assumed that the concentration of insecticides and herbicides in the fish of the sea arises from initial concentration by the photosynthetic organisms which are also the primary source of atmospheric oxygen. The problem is whether the herbicides and pesticides concentrated by the basic photosyn-

Table 1. Measured oxygen abundances, percent by volume (dry air).

Latitude	Longitude	Date collected	Analysis performed during: 15 to 17 April 1969 Value	15 to 17 April 1969 S.D.	9 to 11 April 1969 Value	9 to 11 April 1969 S.D.
		In 1967 by the R.V. Oceanographer				
49°00′N	007° 5′W	4/18			20.9457	±.0006
45°40′N	014°30′W	4/17	20.9463	±.0006		
42°09′N	019°19′W	4/16	20.9450	±.0017		
40°02′N	024°07′W	4/15	20.9450	±.0010		
36°08′N	031°08′W	4/14	20.9460	< ±.0001		
35°54′N	018°12′E	5/6	20.9460	< ±.0001		
35°54′N	018°12′E	5/6	20.9453	±.0012		
21°00′N	038°30′E	5/22	20.9460	< ±.0001		
21°00′N	038°30′E	5/22	20.9457	±.0006		
19°20′N	070°53′E	6/14			20.9427	±.0023
19°10′N	068°20′E	6/18			20.9487	±.0006
19°10′N	068°20′E	6/18			20.9440	±.0020
18°54′N	065°00′E	6/19			20.9450	±.0026
14°27′N	070°12′E	6/5			20.9487	±.0042
11°06′N	060°04′E	6/3			20.9493	±.0059
10°00′N	092°04′W	11/17	20.9460	±.0010		
10°00′N	092°04′W	11/17	20.9457	±.0006		
08°09′N	073°06′E	7/2			20.9457	±.0058
05°08′N	090°01′E	7/6	20.9463	±.0006		
00°49′N	092°00′W	11/22	20.9457	±.0006		
00°00′	106°10′E	8/10	20.9460	< ±.0001		
05°12′S	113°16′E	8/12	20.9463	±.0006		
09°52′S	115°06′E	8/14	20.9463	±.0006		
10°01′S	084°59′W	11/16	20.9463	±.0006		
15°02′S	113°01′E	8/17	20.9463	±.0006		
15°02′S	113°01′E	8/17	20.9437	±.0032		
20°00′S	115°01′E	8/18	20.9457	±.0006		
20°00′S	076°54′W	11/9	20.9463	±.0006		
25°20′S	112°01′E	8/20	20.9457	±.0006		
30°00′S	075°00′W	11/4	20.9457	±.0006		
30°25′S	114°50′E	8/23	20.9453	±.0012		
32°18′S	130°42′E	9/4			20.9450	±.0017
32°18′S	130°42′E	9/4	20.9453	±.0006		
34°16′S	149°58′W	10/18			20.9480	±.0020
35°00′S	165°00′W	10/15			20.9450	±.0061
35°00′S	135°00′W	10/20			20.9473	±.0012
35°00′S	135°00′W	10/20			20.9467	±.0015
37°42′S	180°00′W	10/12			20.9470	±.0010
Unknown	Unknown	Unknown			20.9457	±.0006
Unknown	Unknown	Unknown	20.9460	< ±.0001		
Unknown	Unknown	Unknown	20.9463	±.0006		
Unknown	Unknown	Unknown	20.9457	±.0006		
Unknown	Unknown	Unknown	20.9457	±.0006		
Unknown	Unknown	Unknown			20.9487	±.0006
Unknown	Unknown	Unknown			20.9457	±.0012
Unknown	Unknown	Unknown			20.9453	±.0012