brief communication

# Effects of physiologically relevant pressures of helium on the structure of cholesterol-containing lipid bilayers

## A neutron diffraction study

N. P. Franks and W. R. Lieb

Biophysics Section, The Blackett Laboratory, Imperial College of Science, Technology and Medicine, Prince Consort Road, London SW7 2BZ, United Kingdom

ABSTRACT We have used neutron diffraction to study the effects of helium gas (1–210 atm) on the structure of a lipid bilayer model of neuronal plasma membranes. We have recorded diffraction patterns from hydrated multilayers of dimyristoyl lecithin and 40% (molar) cholesterol to a resolution of ~6.5 Å and have calculated scattering amplitude density distributions as a function of pressure. We find that there are no significant changes in the scattering density profiles at 95% confidence over the range of pressures investigated, suggesting that the physiological effects of high helium pressure are unlikely to be a consequence of changes in the structures of the lipid bilayer portions of membranes.

## INTRODUCTION

The physiological effects of high pressures of helium are of interest for two main reasons. (*a*) Helium has been used extensively in gas diving mixtures as a partial or total replacement for nitrogen, whose narcotic effects at high pressures would otherwise restrict the depths which can be reached by human divers. Using helium/oxygen mixtures (Heliox) or helium/oxygen/nitrogen mixtures (Trimix), pressures up to ~60 atm have been tolerated by human divers for periods of days (Bennett and McLeod, 1984; Hempleman et al., 1984). However, even with these helium-based gas mixtures, dives are restricted by a complex range of adverse behavioral effects (Brauer et al., 1969; Halsey, 1982) collectively known as the high pressure neurological syndrome (h.p.n.s.). (*b*) High pressures (in the order of 100–200 atm) of helium can reverse general anesthesia in air-breathing animals such as mice (Lever et al., 1971), and many studies have been carried out with a view to determining the molecular basis of the pressure/anesthetic antagonism and thus elucidating the mechanisms underlying general anesthesia.

Because of the central role that membranes play in nervous conduction, the effects of pressure on simple model membranes have been extensively studied (for a review, see Macdonald, 1984), although there has been very little work using direct structural methods such as x-ray and neutron diffraction. Most of the diffraction work that has been done has concentrated on the effects of high hydrostatic pressures on lipid-phase transitions in cholesterol-free bilayers (Stamatoff et al., 1978; Utoh and Takemura, 1985; Braganza and Worcester, 1986*a*; Shyamsunder et al., 1989; Winter et al., 1989), although one report (Braganza and Worcester, 1986*b*) included pressure effects on cholesterol-containing bilayers. Because the physiological effects of high pressures of helium are known to be different to those of high pressure per se (Macdonald, 1975; Dodson et al., 1985), there may also be differences in their effects on membrane structure, yet we know of no structural work that has been reported on the effects of physiologically relevant pressures (1–200 atm) of helium on the structure of lipid bilayers. In addition, cholesterol-free bilayers are poor models of the cholesterol-rich bilayer regions of neuronal plasma membranes, so it is also important to use cholesterol-containing bilayers. For these reasons, we have studied the effects of helium pressures from 1 to 210 atm on a cholesterol-containing bilayer model of neuronal plasma membranes.

## MATERIALS AND METHODS

### High-pressure neutron diffraction chamber

A neutron diffraction chamber (see Fig. 1*A*) capable of working at 400 atm helium pressure was fabricated from a single piece of hafnium-free zirconium alloy (zirconium 20 supplied by Imperial Metal Industries Plc., Birmingham, UK). The internal volume was ~75 $cm^3$ and the wall thickness over the beam area was 4.7 mm. Approximately 26% of the main beam was absorbed by the chamber and, to reduce background scatter from the back of the chamber, the main beam was absorbed after the specimen by a sheet of cadmium.

### Methods

Data collection and analysis were essentially as described by Worcester and Franks (1976) and Franks and Lieb (1979). The measurements were made (in August and September, 1977) at the fission reactor PLUTO at AERE, Harwell, Oxfordshire, UK using a small angle diffractometer modified from Haywood and Worcester (1973). The intensity of the incident beam was continuously monitored by a fission



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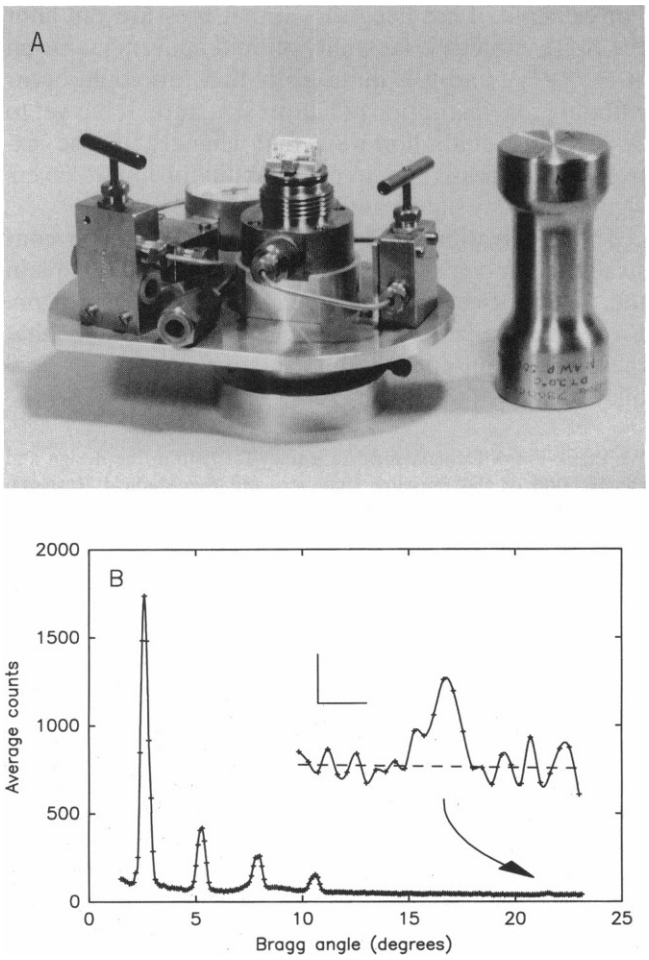


FIGURE 1 (*A*) High-pressure neutron diffraction chamber used in the experiments. The top of the chamber (*right*) has been unscrewed and the glass slide holding the multilayer specimen is visible. The two high-pressure valves were used to admit and release the helium gas. (*B*) A typical neutron diffraction pattern from a multilayer specimen of dimyristoyl lecithin and 40% (molar) cholesterol at 130 atm of helium. The ordinate represents the average number of counts per scan over each diffraction peak. Each peak was scanned between 4 and 8 times. The inset shows the weak eighth order; the calibration bars represent 0.5 degrees and 5 counts. The lamellar spacing $D$ was 51.6 Å.

chamber detector. Lamellar diffraction patterns were recorded using $\theta$–$2\theta$ scans and mosaic spreads were determined using rocking curves. Repeated $\theta$–$2\theta$ scans were averaged, and the observed intensities $I_{obs}(h)$ were obtained by summing the counts under the Bragg peaks after background subtraction. The observed intensities were corrected for specimen absorption and geometrical factors related to the diffraction geometry exactly as described by Franks and Lieb (1979). Structure factors (the square root of the corrected intensities) were assigned phase angles (0 or $\pi$) as previously determined by Franks and Lieb (1979). The neutron wavelength (4.73 Å) was determined using a bismuth standard. Data were collected at 23 ± 1°C. The helium gas used for pressurizing the chamber was analyzed by gas chromatography and found to have less than 0.31 parts per million of hydrocarbon contamination, of which 70% was methane.

Oriented multilayer samples (typically 20 μm thick) were prepared (Worcester and Franks, 1976) on glass microscope slides from a mixture of dimyristoyl lecithin (DML) and 40% (molar) cholesterol (both from Sigma Chemical Co., Poole, Dorset, UK) and were hydrated at 75% relative humidity. Rocking curves showed that the full-width, half-maximum mosaic spread of the multilayer samples was typically <1°, and this did not change significantly as a function of pressure.

## RESULTS AND DISCUSSION

A typical neutron diffraction pattern is shown in Fig. 1 *B*. As previously described (Franks and Lieb, 1979), DML/cholesterol bilayers at ambient pressures give eight orders of diffraction corresponding to a resolution of ~6.5 Å, with orders 5–7 being not significantly different from zero. The calculated structure factors were essentially identical to those previously published (Franks and Lieb, 1979) so the phases could be directly assigned. The calculated scattering amplitude density distribution for the control (1 atm helium) bilayer is shown as the solid line in the top part of Fig. 2. We found that at 130 and 210 atm of helium gas there were no detectable changes in bilayer structure at the 95% confidence level. This is shown in Fig. 2, where on the control profile we have superimposed the scattering density profiles (calculated at 2 Å intervals) obtained in the presence of 130 and 210 atm of helium.

In the lower part of Fig. 2 the dashed lines represent the error envelope (at 95% confidence) for the control profile determined using the methods outlined in detail

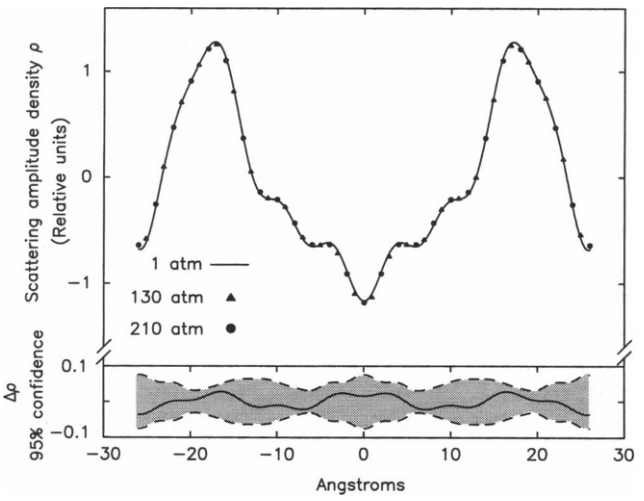


FIGURE 2 (*Top*) Scattering amplitude density profiles for a DML/cholesterol bilayer at 1 atm of helium (*solid line*) and at 130 atm (▲) and 210 atm (●) of helium. (*Bottom*) The dashed lines represent the 95% confidence limits for the control profile (at 1 atm helium) and the solid line is the difference in scattering amplitude density between the control profile and the profile at 210 atm pressure.

in Franks and Lieb (1979). The solid line is the difference between the scattering profile for the control and that obtained at 210 atm, the highest pressure we used. This difference clearly lies within the 95% confidence limits for the control profile and shows that structural changes in these lipid bilayers at physiological pressures of helium are very small indeed.

We did observe a small, but barely significant ($P = 0.12$, $n = 5$), increase in the lamellar spacing $D$ at 210 atm. Taking account of the precision in our determination of $D$, we estimate that the magnitude of the overall compressibility $-(1/\Delta P)(\Delta D/D)$ of the multilayer stack in the direction perpendicular to its plane is less than $\sim -2 \times 10^{-5}$ atm$^{-1}$. This value is comparable to estimates that can be made from hydrostatic pressure measurements on liquid-crystalline bilayers, both with and without cholesterol, which range from $\sim -1.5 \times 10^{-5}$ to $-4.8 \times 10^{-5}$ atm$^{-1}$ (Stamatoff et al., 1978; Braganza and Worcester, 1986*b*). It seems clear that any additional or compensating structural changes due to the presence of helium per se must be very small.

There have been numerous studies on the effects of hydrostatic pressure on lipid bilayers using a variety of techniques. Most studies (e.g., Liu and Kay, 1977; Mountcastle et al., 1978; Kamaya et al., 1979; for a review, see Macdonald, 1984) have focused on the shift in the main chain-melting phase transition temperature in cholesterol-free bilayers, which typically increases by $\sim$0.02°C/atm. Consequently, if nerve membranes were poised close to such a phase transition, an increase in pressure could result in a substantial alteration of bilayer structure. It seems unlikely, however, that these observations have any direct physiological significance, because a small decrease in temperature should have an equivalent effect; this is not observed even in cold-blooded animals where body temperatures can be changed by several degrees without obvious effects. (A similar argument can be used against the physiological relevance of the very small changes in lipid bilayer fluidity that are observed at high pressures [Boggs et al., 1976; Finch and Kiesow, 1979; Mastrangelo et al., 1979], because these can also be mimicked by a small change in temperature.) Moreover, nerve plasma membranes not only possess a highly heterogeneous mixture of diacyl lipids but also contain a high concentration of cholesterol. Because cholesterol is known to effectively abolish the chain-melting phase transition in simple lipid systems, it seems unlikely that such phase transitions occur in nerve plasma membranes under physiologically relevant conditions.

It remains possible, of course, that the small changes that must occur in lipid bilayers at pressure have some important physiological consequences, even though they might not be observable using diffraction techniques. For example, it has been shown that pressure can alter the passive ionic permeability of lipid bilayers (Johnson et al., 1973), and it is quite likely that this could occur without gross disruption of bilayer structure. It has yet to be demonstrated, however, that changes in the extremely low passive ionic permeability of lipid bilayers would have any significant effect on nerve activity.

The observations that we report here put clear constraints on models which might be proposed to explain the physiological effects of high pressures of helium. From the available data, it seems most unlikely that these are a consequence of structural changes in lipid bilayers.

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