
The Effects of Pressure on the Molecular Structure and Physiological Functions of Cell Membranes

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References

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The effects of pressure on the molecular structure and physiological functions of cell membranes

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The effects of high pressure on the phase state and molecular structure of pure lipid bilayers are discussed. The relations of ΔH , ΔS and ΔV in phase transitions are straightforward and are discernible in heterogeneous bilayers in natural membranes. The effects of pressure on the dynamic properties of bilayer constituents are less clearly understood, but order parameters obtained at pressure by different techniques show agreement. The extent and significance of hydration is poorly understood.

Four physiological functions are discussed: passive permeability, active transport, membrane excitability and synaptic transmission. It is shown that a full interpretation of the kinetic effects of pressure on these processes requires much more detailed molecular information than is available at present.

INTRODUCTION

The study of life under high pressure began in the nineteenth century, in France (Regnard 1891). Much of the pioneering work in both the nineteenth and early part of the twentieth centuries had to do with the motor activity of animals (Regnard 1887; Ebbecke & Schaefer 1935; Cattell 1936). The study of the colloidal properties of cytoplasm, its contractility and structure under pressure was a significant contribution to cell physiology in the period 1930–70 (Marsland 1938, 1970; Kitching 1957). During the past decade, interest in the structure and function of membranes under high pressure has grown rapidly. Although a significant symposium was held in 1971 on ‘The effects of pressure on organisms’ (Sleigh & Macdonald 1972) it contained no contributions dealing specifically with membranes, but now high pressure is recognized as a useful thermodynamic and kinetic variable in membrane research. Pressure is also better appreciated as a formidable factor in human diving and in the physiology of marine organisms generally.

This paper answers the broad question ‘how does pressure affect cell membranes?’ by first discussing the properties of lipid bilayers under high pressure. It then proceeds to a discussion of some physiological properties of cell membranes under pressure: passive permeability, active transport, excitability and synaptic transmission. The experimental pressure range of interest is generally within 1–1000 atm (about 0.1 to 100 MPa or 1–1000 bar), which also corresponds to the pressure range occupied by organisms in the oceans.

1. LIPID BILAYERS

1.1. *Introduction*

There are two primary mechanisms by which high hydrostatic pressure affects the molecular organization of lipid bilayers. The best understood is bulk compression, in which the

intermolecular distances between acyl chains within the bilayer are reduced, although not uniformly. The second mechanism involves a change in hydration and is better understood in proteins. For bilayer lipids, it is likely that the volume of the bilayer–water system is reduced by an increase in the packing density of solvent water around the lipid headgroups. The structure of bilayers under pressure will be discussed under two headings; phase state and molecular structure.

1.2. Phase state

Bilayers composed of a single phospholipid species exhibit marked thermotropic phase changes that, in attenuated form, are also manifest in heterogeneous natural membrane bilayers, unless obscured by the presence of cholesterol or proteins. The crystalline–liquid–crystalline phase transition of pure phospholipid bilayers involves a well known positive enthalpy change and also positive volume and entropy changes. At equilibrium $\Delta H = T \Delta S$, in which T is the transition temperature. From Le Chatelier we may anticipate that the volume increase that occurs on passing to the liquid–crystalline state will require a higher temperature to complete the transition at elevated pressure. At constant temperature we may predict that the application of sufficient pressure will cause an isothermal transition to the crystalline (gel) state.

The source of the volume change in the main transition in phospholipid bilayers is a loosening in the packing of the acyl chains, with an unknown but minor contribution from headgroup hydration (Nagle & Wilkinson 1978). So ΔH and ΔV are comparable to, but smaller than, the ΔH and ΔV in the melting of alkanes and liquid crystals. Other thermotropic structural transitions occur in bilayers, such as the pre-transition in DPPC liposomes and the less well defined structural changes such as lipid cluster formation in natural bilayers (Lee *et al.* 1974). The thermodynamics of the pre-transition cause it to be less sensitive than the main transition to high pressure (reviewed in Wann & Macdonald 1980); for lipid cluster formation there is, as yet, no information.

The phase behaviour of bilayers under high pressure has been studied with a wide variety of techniques, conveniently classified as (a) thermodynamic and (b) spectroscopic. The Clausius–Clapeyron relation

$$dT/dP = \Delta V T / \Delta H, \quad (1)$$

which expresses the increase in transition temperature T with increase in pressure, applies not only to the phase transitions in model bilayers but also to the less sharply defined transition in natural membrane bilayers.

(i) Model membrane bilayers

(a) *Thermodynamic methods.* The main transition in dipalmitoyl phosphatidyl choline (DPPC) and other phospholipid liposomes has been measured at high pressure by differential scanning calorimetry and dilatometry, which demonstrates that ΔH and ΔV are constant over 136 atm† and 400 atm respectively (Mountcastle *et al.* 1978; Liu & Kay 1977; Macdonald 1978) (table 1). The cooperativity of the transition is also unaltered. High pressure dilatometry shows the bulk compressibility of DPPC multilamellar liposome bilayers in pure water to be 0.1 ml mol atm⁻¹ above the transition and at least an order of magnitude less below (Liu & Kay 1977).

It is clearly of interest to reconcile ΔV at the main transition with the other thermodynamic

† 1 atm = 101 325 Pa.

data (ΔH , ΔS) and with the estimates of the numbers of rotational isomers that might exist in acyl chains. Trauble & Haynes (1971) attempted this exercise but unfortunately their value for ΔV for DPPC bilayers was one third of the true value. Nevertheless, the corrected value of ΔV improves their point concerning the relation between ΔV and ΔS in the bilayer transition and the alkane melting process. When expressed per CH_2 group, ΔV and ΔS for the bilayer

TABLE 1. PRESSURE, VOLUME AND ENTHALPY RELATIONS IN THE PHASE TRANSITION OF A PURE PHOSPHOLIPID BILAYER AND A NATURAL MEMBRANE BILAYER

DPPC main transition, multilamellar bilayers			
$\frac{\Delta H}{\text{kcal mol}^{-1}}$	$\frac{\Delta V}{\text{ml g}^{-1}}$	$\frac{dT/dP}{\text{K atm}^{-1}}$	high pressure method for dT/dP
8.6 ⁽¹⁾	0.035 ^{†(5)}	0.0220	spin label ⁽¹⁰⁾
8.6 ^{†(2)}	0.035 ⁽⁶⁾	0.0229	dilatometry ⁽¹¹⁾
8.7 ⁽³⁾	0.033 ⁽⁷⁾	0.0238	dilatometry ⁽¹²⁾
8.6 ⁽⁴⁾	0.036 ⁽⁸⁾	0.0240	calorimetry ⁽¹³⁾
	0.035 ⁽⁹⁾	0.0218	turbidimetry and dynamic laser scattering ⁽¹⁴⁾
		0.0224	turbidimetry ⁽¹⁵⁾
		0.0240	turbidimetry ⁽¹⁶⁾
		0.0275	fluorescence polarization ⁽¹⁷⁾
		0.0208	X-ray (under 20% hydrated only) ⁽¹⁸⁾
		0.0228	(calculated from ΔH and ΔV) [†]
\dagger 8.6 kcal mol ⁻¹ = 11.7 cal g ⁻¹ ; 0.035 ml g ⁻¹ = 25.7 ml mol ⁻¹ .			
<i>Acholeplasma laidlawii</i> B. membrane			
$\frac{\Delta H}{\text{cal g}^{-1} \text{ (lipid)}}$	ΔV		
3.6 ⁽¹⁹⁾	(2.1% of membrane; 4% lipid ⁽²⁰⁾)	0.016	fluorescence polarization ⁽²¹⁾
6 ⁽²²⁾		0.017	turbidimetry ⁽²¹⁾
9.8 ⁽²³⁾		0.03	calculated from ΔH

References: (1) Phillips *et al.* (1969); (2) De Kruffy *et al.* (1973); (3) Mabrey & Sturtevant (1976); (4) Lentz *et al.* (1978); (5) Nagle (1973); (6) Blazyk *et al.* (1975); (7) Liu & Kay (1977); (8) Wilkinson & Nagle (1978); (9) Macdonald (1978); (10) Trudell *et al.* (1974*b*); (11) see (7); (12) see (9); (13) Mountcastle *et al.* (1978); (14) Ceuterick *et al.* (1978, 1979); (15) Kamaya *et al.* (1979); (16) MacNaughtan & Macdonald (1980); (17) Chong *et al.* (1981); (18) Stamatoff *et al.* (1978); (19) Reinhert & Stein (1970); (20) Melchior *et al.* (1977); (21) Macdonald & Cossins (1983); (22) Melchior *et al.* (1970); (23) De Kruffy *et al.* (1972).

transition are approximately half the corresponding value for the alkane melt. There is no difficulty in matching the bilayer transition ΔV with an increase in the number of rotational isomers in the acyl chains; rather the difficulty lies in knowing the type of rotational isomers and hence their volume contribution to ΔV . Estimates of the increase in the number of gauche isomers in the transition of DPPC have been discussed by Gaber & Peticolos (1977).

(*b*) *Spectroscopy.* X-ray diffraction of hydrated DPPC (less than 20% (by mass) water) shows it exists in gel and liquid-crystalline form, with a transition at 60.5 °C. This is discernible as a change in the lamellar periodicity of more than 5.5 nm, which is characteristic of the gel state, to a periodicity over 5.3 nm in the liquid-crystalline state. Over a pressure range of 875 atm (Stamatoff *et al.* 1978), $dT/dP = 0.0208 \text{ K atm}^{-1}$. Pressure increases the lamellar periodicity of the liquid-crystalline state at 78 °C from approximately 4.94 nm at normal pressure to 5.22 nm at 800 atm, or 0.007% atm⁻¹ over 800 atm. In the gel phase (26 °C) the corresponding change is *minus* 0.0007% atm⁻¹, of which a quarter is due to the isotropic compression of interlamellar water.

These changes in periodicities mean that the liquid-crystalline bilayer compresses laterally

and expands transversely, actually increasing in thickness as the acyl chains become more ordered under pressure. Despite the low water content of the DPPC bilayers it is reasonable to extend these dimensional changes to the more familiar fully hydrated type of bilayer. It appears that increased pressure orders a bilayer in a manner similar to the influence of intercalated proteins or cholesterol.

The well established spectroscopic methods for monitoring the phase behaviour of lipid bilayers have been used at high pressure; electron spin resonance measurements of Tempo partitioning (Trudell *et al.* 1974*a, b*; Plachy 1976), fluorescence polarization of DPH (Chong *et al.* 1981), Raman spectroscopy (Wong *et al.* 1982) and most simple of all, light scattering (Ceuterick *et al.* 1978; Kamaya *et al.* 1979; MacNaughtan & Macdonald 1980). In general the results are consistent with the Clausius–Clapeyron relation (table 1). No change in the cooperativity of the transition is reported from these spectroscopic studies.

The data in table 1 give the impression that bilayer phase transitions are relatively insensitive to pressure. However, it may be predicted that a bilayer with a highly cooperative transition will be drastically affected by a moderate pressure, well within the human diving range of 50 atm, if it is applied at the transition temperature. Barkai *et al.* (1983) have confirmed this prediction by using the fluorescence polarization of DPH to monitor bilayer fluidity, and so too have other workers employing ‘pressure-jump’ techniques to study the kinetics of the transition process (Clegg *et al.* 1975; Gruenwald *et al.* 1980; Yager & Peticolas 1982). A recent and elegant example is the pressure-jump or kinetic-calorimeter apparatus described by Johnson *et al.* (1983), which measures the heat changes in a sample subjected to pressure steps of 4 atm. The kinetics of the bilayer transition, melting or freezing, are slow, being rate determined by a nucleation process that is measured in seconds.

One of the main gaps in our knowledge of how pressure affects bilayer phase transitions is the part played by the lipid headgroups and their electrostatic or hydrophobic interactions with solvent water. Significantly perhaps, some of the properties of the negatively charged dipalmitoyl phosphatidic acid (DPPA) bilayer under pressure have proved particularly interesting.

In most bilayers whose transition temperatures have been lowered by the inclusion of general anaesthetics, inert gases or other membrane soluble substances, dT/dP is independent of pressure (Mountcastle *et al.* 1978; Macdonald 1978; Kamaya *et al.* 1979; MacNaughtan & Macdonald 1980). The phase behaviour of DPPA monitored by the spin probe TEMPO provides an interesting exception (Galla & Trudell 1980*a*). The rate of change, dT/dP , for the untreated bilayers is 0.015 K atm^{-1} . However, in bilayers treated with methoxyflurane sufficient to reduce the onset of melting in the phase transition by 20°C and broaden the ‘width’ of the transition, dT/dP is 0.085 K atm^{-1} , over the pressure range 1–50 atm. Above 50 atm, dT/dP resumes a value close to that of the untreated bilayer. Furthermore, 100 atm pressure restores the transition width to normal, unlike that for DPPC or DMPA bilayers treated with a methoxyflurane (Macdonald 1978; Galla & Trudell 1980*a*). The simplest explanation for these effects is that pressure decreases the bilayer–water partition coefficient of methoxyflurane, effectively ‘dissociating’ it out of the bilayer over the 1–50 atm pressure range. This implies a very large decrease in the partial molar volume of methoxyflurane as it moves from the bilayer to the aqueous phase. By treating the partitioning of methoxyflurane between water and bilayer as a binding constant K , then from $d(\ln K)/dP = -\Delta V/RT$ it may be shown that for 100 atm to cause a 50% change in K , V needs to be 100 ml mol^{-1} . This is 8 times greater than the

difference between the partial molar volume of methoxyflurane in water and in lecithin-cholesterol bilayers (Kita *et al.* 1981). The decrease in the cooperativity of the DPPA phase transition that methoxyflurane causes implies that the anaesthetic disorganizes the bilayer significantly, probably with a large volumetric effect. Perhaps a full explanation will involve the distinctive properties of TEMPO in addition to the peculiar volumetric properties of the DPPA-methoxyflurane system-water system.

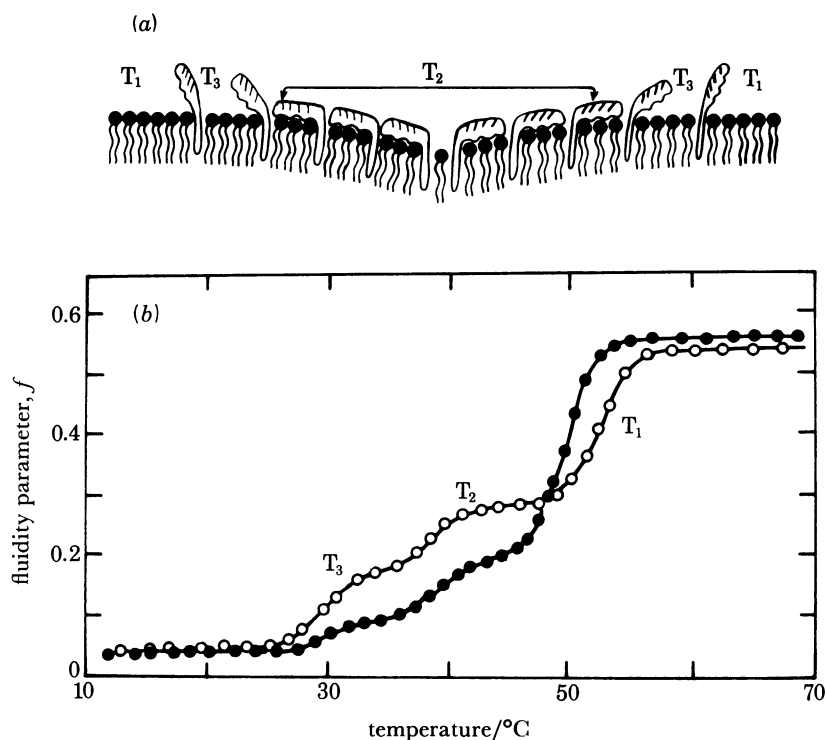


FIGURE 1. (a) Polymyxin-phosphatidic acid bilayer interaction, after Galla & Trudell (1980*b*). T_1 , unaltered bilayer; T_2 , domain of polymyxin bound hydrophobically and electrostatically to bilayer; T_3 , hydrophobically bound polymyxin surrounding T_2 . (b) Effect of pressure on fluidity parameter of polymyxin (4 mole %) - phosphatidic acid bilayers. ●, 1 atm; ○, 100 atm. The inflexions T_1 , T_2 , T_3 correspond to the phase transitions of the domains in (a). Note that only T_1 is increased in temperature by pressure, but the amplitude of the transitions of T_2 and T_3 are increased. Modified from Galla & Trudell (1980*b*).

DPPA bilayers also bind the antibiotic cyclic peptide polymyxin, whose hydrophobic 'tail' inserts between the polar lipid headgroups and fluidizes the bilayer. Figure 1 illustrates the three resultant domains postulated by Galla & Trudell (1980*b*) in their interpretation of how pressure affects the phase state of the DPPA-polymyxin system. The spin label TEMPO provides a profile of the phase transition, which under 100 atm pressure, undergoes two main changes. The binding of lipid by polymyxin is enhanced, particularly the hydrophobically bound form, T_2 , seen as a shift up the fluidity axis in the transition profile in figure 1. The unmodified DPPA domain shows an increase in its transition temperature.

(ii) *Natural membrane bilayers*

The thermodynamics of the phase transition in the membrane bilayer of *Acholeplasma laidlawii*, a sub-bacterial prokaryote, are broadly consistent with the Clausius-Clapeyron relation. If the

transition temperature is taken to be 35 °C, a value of 0.04 ml g⁻¹ lipid for ΔV , $\Delta H = 9.6 \text{ cal g}^{-1}\dagger$, then $dT/dP = 0.03 \text{ K atm}^{-1}$ (table 1). Despite the low cooperativity of the transition, the heterogeneity of the lipids involved and the presence of membrane proteins, DPH fluorescence polarization and light absorption methods independently show dT/dP to be 0.017 K atm^{-1} (Macdonald & Cossins 1983).

In contrast to *Acholeplasma*'s membrane, which has little cholesterol, the human erythrocyte membrane is rich in cholesterol and consequently lacks a clear endothermic phase transition, but it does exhibit a number of thermotropic structural changes. Pyrene excimer fluorescence, which is determined by lateral diffusion within the bilayer, is alleged to reveal such a thermotropic change in ghost membranes at 19 °C, in the form of a discontinuity in an Arrhenius plot of excimer–monomer fluorescence intensity (Flamm *et al.* 1982). At 450 atm, the discontinuity becomes more convincing at a temperature of 29 °C, which indicates the presence of a structural transition with $dT/dP = 0.022 \text{ K atm}^{-1}$. A slightly greater value ($0.027 \text{ °C atm}^{-1}$) is seen in liposomes made from the erythrocyte lipids. Evidence of another pressure-sensitive thermotropic change comes from rabbit lung macrophages equilibrated with stearic acid spin labelled in the C16 position. The rotational correlation time (τ_c) increases at characteristic pressures and temperatures, which indicates a transition to an anisotropic state near the spin label within the interior of the bilayer. In view of the different compositions of plasma and intracellular membranes, it is somewhat surprising that such an abrupt change is detected in whole cells. It is reassuring that a membrane pellet from lysed labelled cells contains all the spin label signal of the intact cells. The dT/dP for the change in τ_c was estimated at 0.027 K atm^{-1} , which implies a high value for ΔV relative to ΔH in the lipid bilayer transitions (Gause *et al.* 1974).

The final example of isothermal transitions caused by pressure in natural membranes is the inner membrane of rat liver mitochondria, which exhibits both a lateral phase separation and a change in permeability under pressure (Wattiaux-De Coninck *et al.* 1977, 1980; Bronfman & Beaufay 1973). (The change in permeability is discussed in §2.2(i).) Interestingly, the phenomenon was discovered in membranes subjected to hydrostatic pressure generated by centrifugation. The phase separation caused by pressure is counteracted by elevating the temperature. So rat liver mitochondria exhibit a marked phase separation in their membrane at -15 °C at normal atmospheric pressure. At 0 °C, 1500 atm causes a comparable phase separation, as judged from freeze–fracture electron micrographs, but if the same pressure is applied at 10 °C no phase separation occurs. So dT/dP is very roughly 0.01 K atm^{-1} , similar to the value for the phase transition in *Acholeplasma* membranes.

1.3. Molecular structure

(i) Static order in model and natural membrane bilayers

Spectroscopic methods have been used at high pressure to quantify the order of bilayer lipids. Three spin label studies have been reported (table 2). Egg phosphatidyl choline–cholesterol bilayers (mass ratio 9:1) labelled with 10-doxyl stearic acid–phosphatidyl choline show a linear increase in order parameter S of 0.02 from 1 to 131 atm at 20 °C (Chin *et al.* 1976; Gaffrey & McConnell 1974). The same increase is seen in similar vesicles (molar ratio, PC:cholesterol, 1:1) labelled with 8-doxyl palmitic and 12-doxyl stearic acid and pressurized to 130 atm at

† 1 thermochemical calorie = 4.184 J.

room temperature (Boggs *et al.* 1976). Changes in the order parameter of spin labels situated in different regions of the same membrane bilayer have also been measured with 5-, 12- and 16-doxyl stearic acid, intercalated in human erythrocyte membranes (whole cells) (Finch & Kiesow 1979). At 37 °C, 100 atm increases the order parameter of the spin label in the 5-position, i.e. the headgroup region, by 1.6%. The order parameter of the 16-position label, which reports the state of the bilayer interior, undergoes a negligible change. These changes, which are all

TABLE 2. ORDER PARAMETERS OF LIPID BILAYERS AS A FUNCTION OF PRESSURE AND TEMPERATURE

order parameter	bilayer†	(pressure coefficient as the increase in temperature required to offset the effect of pressure) (K atm ⁻¹)	temperature at which pressure coefficient holds/°C
spin label S	phosphatidylcholine- cholesterol labelled C ₁₀ ⁽¹⁾	0.023	20
	phosphatidylcholine- cholesterol labelled C ₈ , C ₁₂ ⁽²⁾		
	human erythrocytes labelled C ₅ ⁽³⁾	0.016	37
Raman order parameter	DPPC ⁽⁴⁾	0.05	either side of transition
fluorescence polarization of DPH; lower limiting anisotropy, r_{∞}	goldfish synaptosomes ⁽⁵⁾	0.013	5–20
		0.017	20–35
fluorescence polarization of DPH; steady state polarization, P	goldfish synaptosomes ⁽⁵⁾	0.013	5–20
		0.018	20–35
	myelin-rich vesicles, fish ⁽⁵⁾	0.013	5–20
		0.018	20–35
deep sea fish liver mitochondria ⁽⁶⁾ <i>A. laidlawii</i> B. plasma membrane ⁽⁷⁾	0.017	5–20	
	0.015	mid-transition	

† References: (1) Chin *et al.* (1976); (2) Boggs *et al.* (1976); (3) Finch & Kiesow (1979); (4) Yager & Peticolas (1982); (5) Chong & Cossins (1983); (6) Cossins & Macdonald (1982); (7) Macdonald & Cossins (1983).

less than 3%, can be appreciated by a comparison with other variables. Within the bilayer there exists a gradient of order in the acyl chains, from high at the headgroup end to low at the terminal methyl group. The effect of 100 atm on the 5-position spin label (above) corresponds to differences in order parameter between typical C8 and C7 positions. The increase in order parameter of 0.02 (above) corresponds approximately to the effect of a reduction of 3 °C.

Raman spectroscopy, probably the most powerful technique in this area, yields parameters quantifying both intra-acyl chain and interacyl chain order, and allows estimates to be made of the number of trans- and gauche-rotational isomers present in the bilayer. The application

of Raman spectroscopy to bilayers under high pressure has been limited to measurements of the general-purpose 'Raman order parameter' (Yager & Peticolas 1980). In DPPC bilayers at temperatures either side of the main phase transition, a pressure of approximately 20 atm increases the Raman order parameter by an amount similar to that caused by a decrease of 1 °C (table 2). It may be estimated that 300 atm applied at the phase transition temperature might diminish the number of gauche isomers per mole from 10 to 6. Multilamellar vesicles subjected to pressure over the 5000 atm range reveal marked changes in Raman spectra, owing, even at the highest pressures, to a reduction in the same intermolecular distances that are affected at lower pressures, rather than to a compression of intramolecular bonds (Wong *et al.* 1982). The ratio of the peak heights of the antisymmetric and symmetric CH₂ stretching bands provides a measure of the interactions between acyl chains, and at 28 °C it increases sevenfold while the pressure increases from 1 to 200 atm, at which pressure the bilayer assumes the gel state. At higher pressures the increase in molecular interactions is less affected by pressure, consistent with the compressibility mentioned in § 1.2(i)(a).

Measurements of the fluorescence polarization of bilayers labelled with DPH provide an order parameter, r_{∞} , the lower limiting anisotropy, which is comparable to the spin-label order parameter (Jähnig 1979). Although DPH fluorescence polarization is often used to estimate bilayer microviscosity, the rotational freedom of the fluophor is confined by the presence of the surrounding acyl chains as much as by their viscous drag. In synaptosomal membranes prepared from goldfish brains r_{∞} , the angular amplitude of the probe motion (θ) and P , the more accessible parameter of polarization, increase nearly linearly over 1–1900 atm (Chong & Cossins 1983). Similar polarization data from the phase transition region of *A. laidlawii* membranes, and liver mitochondria from deep sea fish, are also shown in table 2.

(ii) *Dynamic properties*

Microviscosity is a contentious parameter by which the dynamic aspects of bilayer structure are quantified. The pressure coefficient of the microviscosity of micelles, determined by fluorescence polarization is low; 0.020 cP atm⁻¹† and 0.027 cP atm⁻¹ in sodium dodecylsulphate and hexadecyl-trimethylammonium chloride micelles, respectively (Turro & Okubo 1981). That of DPPC bilayers similarly determined in the mid-transition temperature is 7.4 cP atm⁻¹ (Barkai *et al.* 1983). To characterize the dynamic state of goldfish synaptosomal bilayers under pressure, Chong & Cossins (1983) used the 'wobbling diffusion constant', D_w , for the fluorescent probe DPH. Values of D_w and R , the rotational rate of the fluophor, are shown in figure 2. Interpretation of the data is now described.

Pressure generally reduces the velocities of the bilayer constituents, but the close packing of the acyl chains appears to cause the fluophor, DPH in this case, to undergo an accelerated motion of reduced amplitude. D_w and R vary as a function of pressure in a biphasic manner at intermediate temperatures, quite unlike the near linear changes in the order parameter r_{∞} and related parameters P and θ . This interpretation has to be qualified by the reminder that synaptosomal membranes contain a variety of proteins to which DPH binds, and a heterogeneous lipid composition. It would be interesting to measure D_w and R in pure phospholipid bilayers under pressure. However, the interpretation receives some indirect support from an earlier Raman spectroscopy study of DMPC bilayers under pressure (Wong *et al.* 1982). The width of the 'Vs (C—C)' band is markedly decreased by 800 atm, which indicates that 'chain

† 1 P = 10⁻¹ Pa s.

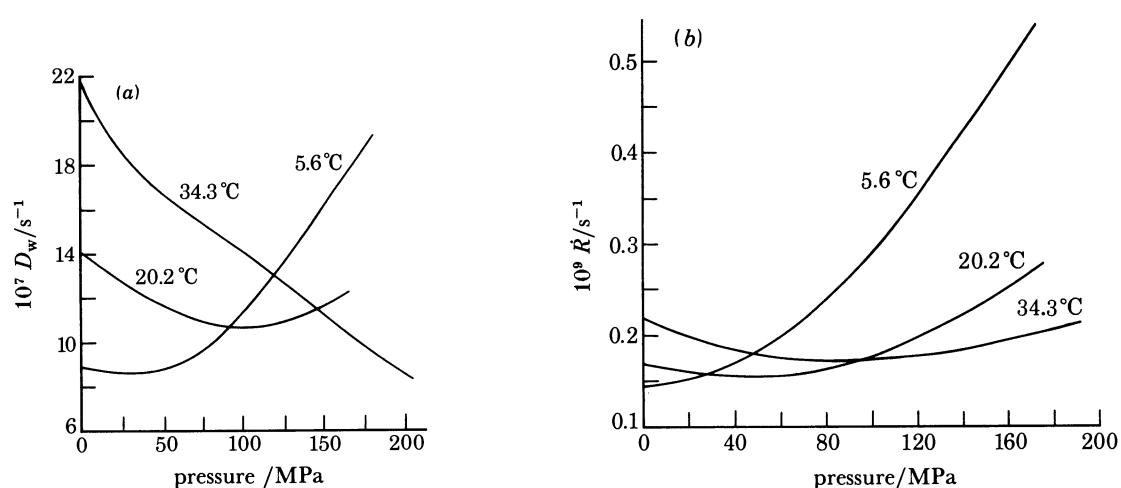


FIGURE 2. Effect of pressure on goldfish synaptosomes. (a) The wobbling diffusion constant. (b) The rotation rate (\dot{R}) for DPH-labelled membranes. Modified from Chong & Cossins (1983).

reorientation is damped' but a further increase in pressure exerts proportionately less effect. This is thought to be caused by the simultaneous increase in order, which, by confining the chains, tends to enhance their velocity. The anisotropic compression of a bilayer therefore appears to cause nonlinear changes in the molecular dynamics of its constituent molecules.

(iii) Hydration

Spin label measurements indicate that pressure increases the polarity of the bilayer in both phospholipid liposomes and erythrocyte membranes (Chin *et al.* 1976; Finch & Kiesow 1979). This is interpreted as an increase in hydration, which is thermodynamically plausible because hydrophobic hydration may involve a negative volume change. One possible consequence of an increase in hydration is a reduction in the motion of the acyl chains. This idea comes from experiments with micelles of ionic surfactant, whose interior microviscosity shows a pressure coefficient between that of a bulk hydrocarbon phase and water (Turro & Okubo 1981). The interior viscosity of such a 'porous' micelle is much reduced by the presence of ions in the aqueous phase, an effect most simply accounted for by the ions altering the water structure within the micelle. Although this idea may not apply to whole bilayers, which are relatively large phases compared to micelles, it may apply to particular regions of a bilayer. In general, pressure is expected to favour the ionization and hydration of polar headgroups, but evidence more direct than that provided by the spin-label experiments is lacking.

2. PHYSIOLOGICAL PROPERTIES OF MEMBRANES

2.1. Introduction

If the properties of lipid bilayers under pressure form the backbone of this paper, then the four physiological processes – passive permeability, active transport, membrane excitability and synaptic transmission – which are discussed in the following sections, are the rudimentary limbs of an embryonic creature. Much of the discussion is based on rate process theory (Johnson *et al.* 1974) and the now substantial body of knowledge dealing with enzyme kinetics and protein structure under high pressure.

2.2. *Passive permeability*

A unidirectional flux may be regarded as a reaction whose rate constant is determined by the free energy of activation in the rate determining step. Solutes diffusing through membranes may find their limiting step is at the interface between the water and membrane, or within the membrane bilayer. In theory, pressure ought to be a useful variable with which to analyse permeation mechanisms and it is clear that pressurized membranes are expected to show altered permeabilities. Three examples are considered here: passive fluxes through model bilayers, through the erythrocyte membrane whose alternative transport pathways are blocked, and the transient fluxes of cations through the voltage sensitive ion channels responsible for action potentials.

(i) *Passive fluxes through model bilayers*

The efflux of K^+ and Na^+ trapped within sonicated liposomes decreases linearly with increase in pressure over a 400 atm range. The liposomes in question were made of DPPC with PA (phosphatic acid) (4%) and of DPPC with PS (phosphatidyl serine) (6%). $\Delta V^\ddagger = 19$ – 20 ml mol^{-1} of ion transported. When the K^+ efflux is mediated by the carrier ionophore valinomycin, the pressure effect remains linear but ΔV^\ddagger is doubled to 37 – 45 ml mol^{-1} (Johnson & Miller 1975). The efflux of glucose is similarly reduced by pressure, with $\Delta V^\ddagger = 37 \text{ ml mol}^{-1}$.

The point of interest here is the relation between ΔV^\ddagger and the structure of the bilayer. By using the Trauble 'kink' hypothesis of permeation, Johnson & Miller (1975) point out that solutes such as K^+ or glucose would fit into the voids created by small kink isomers, but valinomycin would require a larger ($0.8 \text{ nm} \times 0.4 \text{ nm}$) void. It is significant that ΔV^\ddagger for the valinomycin-mediated K^+ efflux is twice that of the unassisted flux. There are several difficulties in applying the Trauble hypothesis, chief of which was recognized by Trauble (1971) and also by Johnson & Miller (1975), namely the estimate of the volumes of kink isomers. These are known for solid hydrocarbons but not for the more fluid interior of a bilayer. Furthermore, the rate-determining step in cation efflux is thought to be the water–bilayer interface rather than the bilayer interior.

High pressure may introduce new rate-determining steps in transbilayer fluxes. Passive permeability is increased when a bilayer is brought to its phase transition temperature, owing to phase separation and the mismatch between phases. By bringing the phase transition temperature close to the experimental temperature, high pressure could conceivably increase bilayer permeabilities. This would not apply to the solute fluxes just discussed, but it accounts for the increase in sucrose permeability seen in rat liver mitochondria subjected to pressures in the range 1–1500 atm (Wattiaux-De Coninck *et al.* 1980; § 1.2(ii)).

(ii) *Passive fluxes through the erythrocyte membrane, whose alternative transport pathways are blocked*

Human erythrocytes treated with ouabain to block active transport, and with bumetamide to block co-transport, provide a membrane barrier through which the diffusion of Na^+ and K^+ may be readily measured. Influx of both these cations is markedly increased by high pressure at 37°C (Hall & Macdonald 1980; Hall *et al.* 1982). A pressure of 100 atm increases K^+ influx by 20%, and over the 100–400 atm range a fivefold increase occurs (figure 3a). This is conveniently quantified as an apparent activation volume for K^+ influx of $\Delta V^\ddagger = -84 \text{ ml mol}^{-1}$, and it is independent of $[K^+]_0$. The sensitivity of the influx under pressure has two striking

features. It decreases with a reduction in temperature, and at 12 °C it becomes unaffected by pressure. At normal pressure, 12 °C is the temperature at which the erythrocyte K^+ influx reaches a minimum (figure 3*b*). Other structural changes occur in erythrocyte membranes in divers under pressure for several days, notably the discoid-echinocyte transformation (Paciorek *et al.* 1982). Four hundred atmospheres presumably increases the microviscosity of the erythrocyte bilayer and the temperature of thermotropic structural changes, and it is the latter that may participate in the K influx.

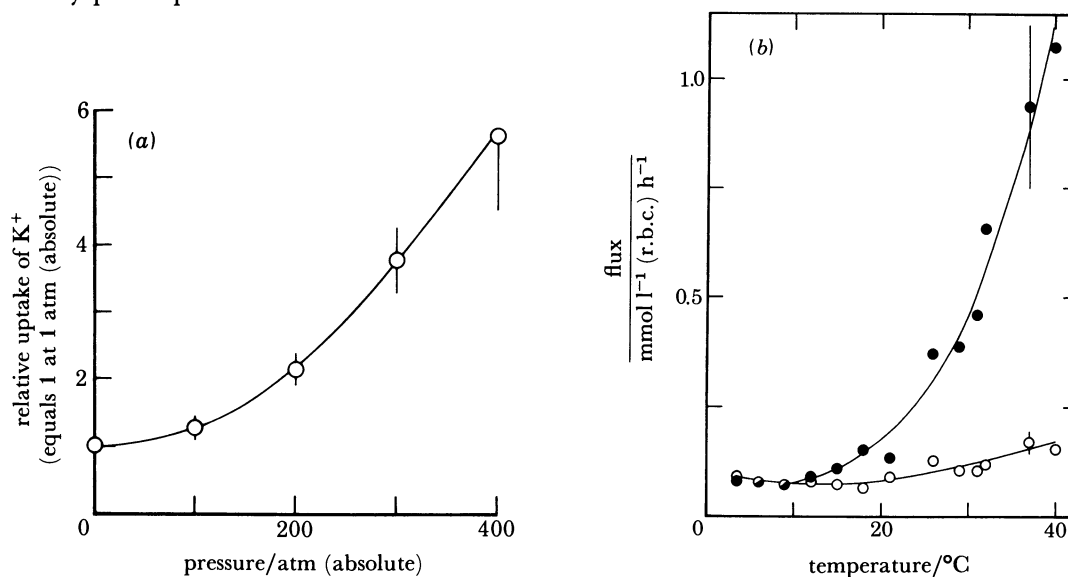
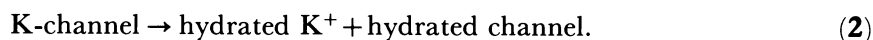
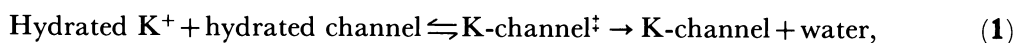


FIGURE 3. Passive permeability of the human erythrocyte membrane to K^+ as a function (a) of pressure at 37 °C and (b) of temperature at normal atmospheric pressure (○) and at 400 atm (●). From Hall *et al.* (1982).

The second feature of note is that ΔV^\ddagger decreases with the substitution of the Cl^- anion present in the external medium by Br^- ($\Delta V^\ddagger = -52 \text{ ml mol}^{-1}$); NO_3^- (-19 ml mol^{-1}) and I^- (-6 ml mol^{-1}). The question is, how can anions affect the ΔV^\ddagger for influx? The following account differs slightly from the interpretation of Hall *et al.* (1982).

In the presence of Cl^- assume that K^+ forms a transitional complex with 'channel' components of the membrane, with a characteristic V^\ddagger .



Reaction (2) is not rate limiting (figure 4). The ΔV^\ddagger arises from the release of water of hydration from both K^+ and the channel, the released water coming from outer hydration shells that comprise ordered and relatively voluminous water. So released water returning to bulk solution containing Cl^- causes a characteristic negative volume change. In a simple equilibrium of this type the overall volume change ΔV will be similar to ΔV^\ddagger , which determines the rate at which the reaction (1) proceeds at different pressures at constant temperature. (In more complex reactions there is often no similarity between ΔV and ΔV^\ddagger .) According to this scheme the anions Br , NO_3 , I affect ΔV^\ddagger either by altering the structure of water in the external solution or by affecting the hydration of the channel. The activation volumes may be equated to n molecules

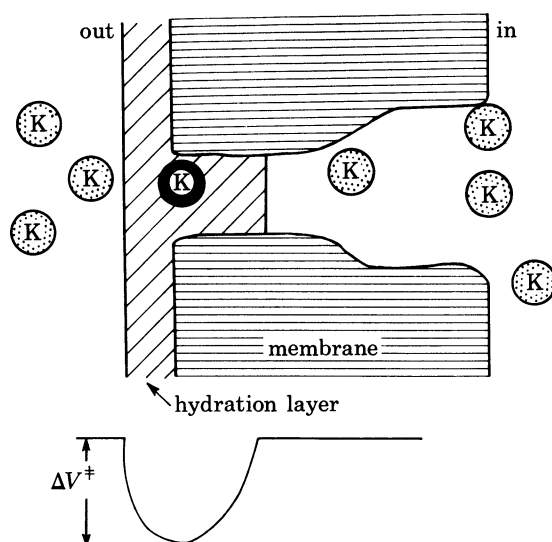


FIGURE 4. Reaction scheme for the passive flux of K⁺ through the human erythrocyte membrane. See text.

of hydrated water being released from the K⁺ and its channel per passage of K⁺ through the membrane. In view of the volume changes known to occur in rearrangements in the structure of water, as in ordinary ice, n is likely to be in the region of 50. This type of speculative interpretation illustrates the detailed level of knowledge that is required to account for the way in which a particular process is modified by high pressure, or of course, by other thermodynamic variables.

(iii) *Passive fluxes of cations through voltage-sensitive ion channels*

The passive flux of K⁺ and Na⁺ through the voltage sensitive channels in excitable membranes is little affected by pressure, whose main effect is to slow the rate of opening of the channels, §2.4(i). Various changes are seen in the input resistance of excitable membranes at high pressure, which imply equally varied changes in their net permeability to ions. Lobster muscle membranes show a transient reduction of 40% at only 10 atm helium pressure, according to Colton & Colton (1982*a*), but Campenot (1975) reports a 50% increase in resistance at 200 atm. *Helix* neurons show a 30% decrease in input resistance at 300 atm (Wann *et al.* 1977). The giant axon from the squid *Loligo vulgaris* undergoes a negligible change in resistance over the range 1–600 atm (Conti *et al.* 1982*a*), but the axon from *Loligo pealii* shows a slight increase at pressures up to 270 atm and a reduction to approximately one half at pressures above 340 atm (Spyropoulos 1957).

2.3. Active transport

The effects of pressure on a variety of active transport processes have been investigated in single cell and epithelial preparations (erythrocytes by Goldinger *et al.* 1980, Hall & Macdonald 1980, Pequeux *et al.* 1980, Van Nice & Caley 1980, Hall *et al.* 1982; bacteria by Paul & Morita 1971, Schlamm & Daily 1971, Berger 1974; frog skin by Brouha *et al.* 1970, Pequeux 1976; fish gill by Pequeux & Giles 1977; crayfish by Roer & Shelton 1982). A variety of membrane pump ATP-ases have also been studied at high pressure. Because of the complexity of the tissues or the multiplicity of the transport processes involved, only a few of these studies

are open to detailed interpretation. The active transport of Na^+ and K^+ under pressure by the human erythrocyte illustrates the importance of defining and controlling all the kinetic variables.

(i) *Membrane ATP-ases*

The pressure sensitivity of the gross Na^+ efflux from human erythrocytes is inhibited by 150 atm only over the range 2–20 meq l^{-1} r.b.c. (Goldinger *et al.* 1980). The slight inhibition (7%) that 69 atm helium pressure exerts on Na efflux is intriguing; it may be the net result of different effects of $p\text{He}$ and hydrostatic pressure (Van Nice & Caley 1980). Ouabain-sensitive Na^+ efflux is reduced by 38% at 150 atm, independent of intracellular ATP-levels (Goldinger *et al.* 1980). Ouabain-sensitive K^+ influx is reduced by 40% at 400 atm in the conditions used by Hall *et al.* (1980) who, consistent with Goldinger's conclusion, found the pump's K_m and V_{\max} for the K^+ influx to be directly reduced by pressure. As an enzyme bound to membrane fragments, the erythrocyte $\text{Na}^+ + \text{K}^+$ -ATP-ase is activated by pressures up to 150 atm, so clearly the pump's vectorial property is an essential feature of its susceptibility to pressure.

Other membrane-bound ATP-ases respond to compression in a manner consistent with their boundary lipid becoming more ordered. The ATP-ases in question are: $\text{Na}^+ - \text{K}^+$ -ATP-ase from the pig kidney medulla; Ca^{2+} -ATP-ase from rabbit sarcoplasmic reticulum; an ATP-ase from *Azotobacter* and from *A. laidlawii* B. (De Smedt *et al.* 1979; Heremans & Wuytack 1980; Ceuterick *et al.* 1978; MacNaughtan & Macdonald 1982). Each ATP-ase shows an Arrhenius plot with a well defined 'break', and for each there is some evidence for supposing that the breaks correspond to a phase transition in lipids associated with the enzyme. This could be a localized phase transition in boundary lipid, or a phase separation process affecting the distribution and lipid environment of the enzyme. In all four enzymes, pressure increases the 'break' temperature of the plots with negligible change in slope. In the order given above, the dT/dP for the Arrhenius break temperatures are: 0.027 K atm $^{-1}$, 0.027 K atm $^{-1}$, 0.020 K atm $^{-1}$ and 0.015 K atm $^{-1}$.

It is reasonable to postulate that pressure affects the activity of these enzymes via the phase state of their associated lipid because these dT/dP values are of the appropriate size and magnitude. For *A. laidlawii* ATP-ase, the dT/dP is the same as that of the transition in the whole membrane (Macdonald & Cossins 1983). However, in the ATP-ase from the kidney and *A. laidlawii*, the addition of benzyl alcohol and pentanol respectively fail to lower the break temperature. This is paradoxical; it implies that the lipid phase that is capable of influencing the enzyme responds to pressure in a thermodynamically orthodox manner, yet is incapable of responding to alcohols in an equally orthodox manner. An alternative explanation is that pressure directly affects the enzyme and some evidence for this comes from experiments in which high speed centrifugation was the source of high pressure (Champey *et al.* 1981). The experiments involve SR vesicles from rabbit muscle, and four properties are monitored after subjecting the vesicles to pressure in the 100–800 atm range by using centrifugation, or in a limited number of experiments, conventional hydraulic compressions. The properties are the ESR spectra from an iodoacetamide spin label, ATP-ase activity, phosphorylation activity and Ca^{2+} transport. If pressurization (centrifugation) occurs at 2 °C in the absence of Ca^{2+} ions, then on restoring the vesicles to a standard buffer at normal pressure, all four properties are irreversibly impaired. If, however, pressurization (centrifugation) is made at 20 °C, or at 2 °C in the presence of Ca^{2+} , or other solutes (for example, 0.3 M sucrose), then the four properties

are not subsequently affected. These results are not inconsistent with the phase transition hypothesis, but the pressure-protective role of Ca^{2+} binding to the SR-ATP-ase makes a more direct effect of pressure on the enzyme plausible. This could take the form of ligand-dissociation or subunit depolymerization, of which many examples exist in the high pressure chemistry of proteins (Heremans 1982; Müller *et al.* 1981; Swezey & Somero 1982).

(ii) *Sensitivity of Na^+ transport to pressure*

The transcellular active flux of Na^+ into the crayfish *Procambarus clarkii* is extremely sensitive to pressure; it is reduced to 20% by 50 atm. Active transport of Na^+ across the isolated frog skin has been measured indirectly at high pressure by means of the short-circuited transepithelial current. 100 atm increases both the current and the transepithelial potential in *Rana temporaria* (Pequeux (1976)), but Woodhouse (1976), who worked with *R. pipiens* skins showed that 100 atm only hyperpolarized the skin and had negligible effect on the short-circuit current. In this case the hyperpolarization is probably due to a diminished Cl^- -shunt (Brouha *et al.* 1970). Segal's (1977) attempt to use a pressure-jump-relaxation technique to investigate the kinetics of Na^+ transport across the skin of *R. pipiens* shows a biphasic current transient that is eliminated by ouabain. This pressure activation of Na^+ transport is presumably a direct effect on the pump, but, of course, the reverse of the inhibition of active transport seen in the erythrocyte.

(iii) *Bacterial transport systems*

Two distinctively microbial experimental contributions are given here. Amino acid transport into the marine bacterium MP-38 is inhibited by pressure, and the following evidence suggests the effect is directly on the transport system. Cells, either pre-incubated with an amino acid (proline or glutamate), or not, are pressurized at 500 atm, during which time labelled amino acid is taken up. For ^{14}C -proline, uptake without pre-incubation is reduced by 500 atm to 1% of the control level, while uptake with prior incubation is only reduced to 40% (Paul & Morita 1971). This is another example of 'pressure protection' by substrate binding and is well worth further investigation.

The use of certain strains of *Escherichia coli* with and without galactoside permease systems enabled Schlamm & Daily (1971) to demonstrate an effect on the transport mechanism. They found that in the strain possessing the galactoside permease, the uptake of β -methyl- ^{14}C -D-thiogalactoside and β -methyl- ^{14}C -galactoside is stimulated by 67 atm helium pressure, whereas in those cells lacking the permease, uptake, presumably passive, is unaffected. The data do not distinguish between hydrostatic pressure and helium pressure, or between a direct effect on the permease and indirect effects on metabolically linked reactions. In the experiments with a marine bacterium, mentioned earlier, Paul & Morita (1971) were able to show that changes in the metabolism of the transported amino acid, caused by pressure, do not affect its uptake.

2.4. Action potentials

(i) *Kinetics*

From measurements made with intracellular recording methods at high pressure on the squid giant axon, *Helix* neurons, frog motor nerve, crayfish ventral cord giant fibres and dog heart Purkinje cells, the generalization has emerged that pressure slows the kinetics of action potentials. Voltage clamp experiments at high pressure have been done to investigate the kinetics of action potentials in the squid giant axon (Henderson & Gilbert 1975; Shrivastav

et al. 1981; Conti *et al.* 1982*a, b*), *Helix* neurons (Harper *et al.* 1981) and frog motor nerve (Kendig 1981). The activation of the Na⁺ current in the squid axon and of the equivalent inward current in *Helix* neurons is slowed by high pressure. The most recent measurements with squid axon use the changes in characteristic rise time of the current, measured as the ratio of peak inward current to the time derivative, and lead to a calculated ΔV^\ddagger at 15 °C of 32 ml mol⁻¹. These data relate to polarizations in the range -20 mV to 10 mV from a holding potential of -80 mV. A greater sensitivity to pressure was seen in the activation of the inward current in *Helix* neurons. ΔV^\ddagger cannot be attributed to a specific reaction because the molecular details of the Na⁺ channel and its gating mechanism are obscure. However, the magnitude of ΔV^\ddagger reported here for the gating (activation) process is within the range expected of conformational changes in proteins or lipid bilayer transitions.

Inactivation of the inward current in *Helix* neurons is reported to be unaffected by pressure (Harper *et al.* 1981). However, in frog nerve (Kendig 1981) and in the most recent work on squid axons, inactivation of the sodium current is shown to be significantly slowed by pressure (Conti *et al.* 1982*a*). This apparent discrepancy may arise from the use of small changes in polarization to determine inactivation in *Helix* neurons. The comparison of the pressure sensitivity of activation and inactivation of the Na⁺ current is particularly interesting and should be pursued.

The kinetics of the early or fast K⁺ conductance in the squid axon are also slowed by pressure (Conti *et al.* 1982*b*). Apparent activation volumes are 31 ml mol⁻¹ at 15 °C and 42 ml mol⁻¹ at 5 °C, for depolarizations of -20 to +20 mV. Technical difficulties obscure the true effect of pressure on the slow K⁺ conductance. In *Helix* neurons, activation of the outward current, equivalent to the K⁺ current in the squid axon, is more sensitive to pressure, and a separate fast transient outward current (I_a) is also slowed by pressure (Harper *et al.* 1981).

These findings raise questions about the way pressure affects 'gating particles' and channels. As was true for membrane-bound enzymes, we find that most of our knowledge of how pressure affects proteins relates to an aqueous solvent, and much has to be learned of hydrophobic solvent-protein interactions under pressure. Channel macromolecules in bilayers should provide an interesting high pressure preparation, but electrical recordings need to be supplemented with spectroscopic and other methods. A preliminary study, made with circular dichroism at pressure, of the polypeptide channel gramicidin A, has been outlined by Harris *et al.* (1976). In its hydrogenated form in ethanol the channel undergoes a significant conformation change at 170 atm, whereas with the channel in a solution of water in ethanol, no effect of pressure is detected. This is tentatively attributed to bulk compression in the first case, and in the second case, the presence of water within the channel pore is thought to render it incompressible.

Voltage clamp experiments have not yet been done to investigate the kinetics of the action potential in pressurized Purkinje cells. Hence the cause of the inhibition by pressure (150 atm) of the maximum rate of depolarization (\dot{V}_{\max}) is not known. The prolonged repolarization phase appears to be caused mainly by a change in the early, rapid events, rather than the terminal repolarization. These primary kinetic disturbances contribute to a slowing of the propagation of the cardiac action potential (Ornhagen & Hogan 1977; Doubt & Hogan 1979).

Action potentials and currents are usually studied at 100 atm or more, in excess of the mammalian diving range, but often effects are seen at lower pressures. A good example is provided by the crayfish ventral cord giant axons, which show reduced rates of depolarization and repolarization at 8 atm or even less (Bryant & Blenkinship 1979).

(ii) *Other excitable properties*

Pressure exerts interesting effects on other excitable properties of neurons. For example, crayfish claw nerves begin to fire spontaneously at pressures in the range 35–200 atm. The frequency of firing is pressure dependent (Kendig *et al.* 1978). Rhythmically firing neurons in *Helix* and *Aplysia* are sensitive to pressure in complex ways. Pacemaker cells in *Helix* suboesophageal ganglia increase their rate of firing some 200% following the application of 200 atm, and the rate subsequently fades to 50% above control level (Wann *et al.* 1979). In *Aplysia*, burster cell activity in the abdominal ganglion shows increased firing frequency within a burst of action potentials, but interburst intervals are lengthened. These effects are apparent up to 700 atm (Parmentier *et al.* 1979).

A notable effect of pressure on an excitable membrane is seen in *Paramecium aurelia*, a ciliated protozoan, whose normal ciliary propulsion is reversed by an inward depolarizing Ca^{2+} current (Otter & Salmon 1979). A pressure of 68 atm blocks the cell's ability to reverse when it swims into a barrier, while decompression causes a transient reversal response. A mutant of *P. aurelia* that has defective Ca^{2+} channels in the ciliary membrane, and consequently no normal reversal response, shows no decompression reversal. These observations suggest that pressure blocks the Ca^{2+} current and that decompression initiates an influx of Ca^{2+} , which is normally triggered by the cell meeting an obstacle.

2.5. *Synaptic transmission*

The move from excitable membranes to their interactions in synapses entails a drastic increase in complexity. As a consequence there is a rich harvest of interesting pressure effects to gather, and a proportionate increase in the difficulty of distinguishing between the direct and indirect effects of pressure. Physiologically, both are probably equally important *in vivo*.

Pressure invariably inhibits synaptic transmission. A particularly pressure-sensitive feature of synaptic physiology is the spontaneous release of acetylcholine from the frog neuromuscular junction (Ashford *et al.* 1982*a*). Figure 5*a* shows that a few atmospheres pressure reduces the frequency of spontaneous transmitter release. The inhibition is reversed on decompression and the sensitivity of the system is unaffected by treatment with hypertonic Ringer or increased K^+ or Ca^{2+} . For the latter, 10 mM Ca^{2+} raises the threshold pressure for the onset of inhibition by 10 atm, thereby providing an interesting case of the reversal rather than the antagonism of pressure. The sensitivity of spontaneous release to pressure may be compared to the inhibitory effect of low temperature, which gives a temperature equivalent of pressure of 0.04 K atm^{-1} . This is higher than the equivalent figures for bilayer phase transitions or microviscosity (table 2).

The post-synaptic membrane in the pressurized frog neuromuscular junction demonstrates striking differences between the growth of the miniature endplate currents (m.e.p.c.) and its subsequent decay, consistent with the view that each is controlled by separate mechanisms. Growth of m.e.p.c. is not significantly affected by pressures up to 150 atm, while the prolonged m.e.p.c. decay phase is broadly consistent with an increase in bilayer microviscosity. Interestingly, the effect is nonlinear (figure 5*b*). The idea that bilayer microviscosity might rate-determine the decay phase has been examined by exposing the preparation to putative bilayer fluidizing agents, such as octanol (Ashford *et al.* 1982*b*). In the presence of an aqueous concentration of 0.1 mM octanol, the m.e.p.c. time constant for decay is reduced, and returns to normal when the octanol is removed. This is apparently a fluidizing effect. Pressurizing the octanol-treated

preparation to 104 atm or more, however, does not restore the time constant to normal; instead a marked reduction occurs. Lesser pressures cause only a slight reduction in the time constant. Qualitatively then, pressure and octanol somehow combine their effects and certainly to not counteract each other. This is not too surprising since the effect of alcohols on m.e.p.c. decay is rather variable, and certainly not proven to involve bilayer fluidity (Wann *et al.* 1980).

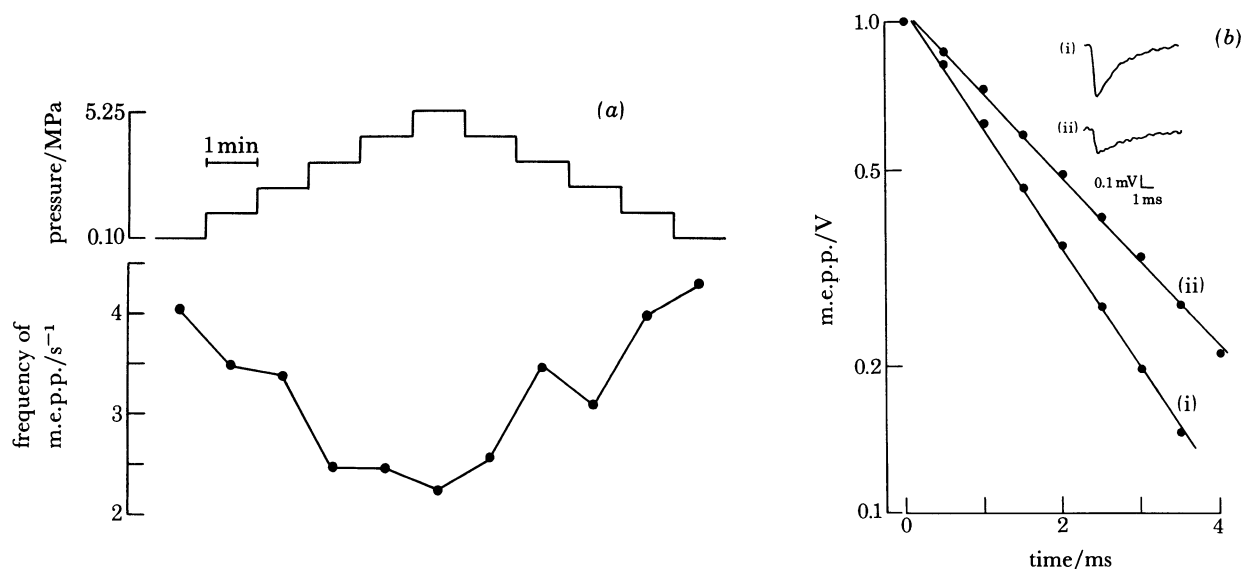


FIGURE 5. Pre- and post-synaptic effects of pressure on the unstimulated motor end plate of frog muscle. (a) The frequency of miniature end plate potentials (m.e.p.p.); (b) the decay of the miniature end-plate potential (i) normal pressure and (ii) at 155 atm (15.55 MPa). The respective time constants for decay are 1.81 and 2.54 ms. From Ashford *et al.* (1982).

Evoked transmitter release at the frog neuromuscular junction is much less affected by pressure than the spontaneous release, and persists at 200 atm. Nevertheless, it is a likely target for inhibition in vertebrate muscles such as the rat diaphragm (Kendig & Cohen 1976), which shows transmission failure at pressures of around 100 atm. In the stretcher muscle in the leg of the lobster a pressure of only 10 atm helium reduces the neuromuscular excitatory junction potential and nearly halves the quantal content. The response to perfused glutamate is not affected by pressure (Colton & Colton 1982*a*). Earlier experiments by Campenot (1975) point to similar pre-synaptic inhibition over the 50–200 atm pressure range. There is also evidence that the release of transmitter at the inhibitory neuromuscular synapse in the lobster muscle is reduced by only 4 atm pressure (Colton & Colton 1982*b*).

Transmission is also clearly inhibited by 100 atm pressure in certain synaptic connections in the abdominal ganglion of *Aplysia*, which are thought to be cholinergic. Two post-synaptic properties, the response to iontophoretically applied acetylcholine and the decay of excitatory post-synaptic potentials (e.p.s.p.) are quite unaffected by 100 atm, while the reduced amplitude of the (e.p.s.p.) and other properties are consistent with an inhibition of transmitter release (Parmentier *et al.* 1981). Other synaptic preparations that have been studied under pressure are the squid giant synapse (Henderson *et al.* 1977), and the rat superior cervical ganglion (Kendig *et al.* 1975; Sauter 1979; Little 1982).

Post-synaptic effects of pressure in evoked synaptic transmission are apparent in a number

of experiments. The application of norepinephrine to isolated duodenum smooth muscle results in a reproducible contraction after a delay. This delay is lengthened in proportion to the applied helium pressure over the 1–30 atm range (Akers & MacCarter 1973). Propranolol and norepinephrine combine to reduce the magnitude of the contraction at 30 atm, whereas when each is applied separately there is no effect. This suggests that pressure affects the intracellular blocking site of propranolol.

Two transmitter binding experiments have been attempted under pressure. In one, a proteoglycolipid extracted from rat gastrocnemius muscle, which binds acetylcholine and BTX, shows remarkable sensitivity to pressure (Taylor & Robertson 1980). For example, 7 atm reduces its ability to bind Ach by 50% in a hydrophobic solvent. Of the two binding sites present, it is the high affinity site that is more sensitive to pressure, and the equilibrium implies a highly cooperative binding process. In another study, reported in preliminary fashion at an early stage, the binding of Ach to *Torpedo* membranes is reduced by 30% on application of 300 atm (Sauter *et al.* 1981).

3. GENERAL CONCLUSION

The four aspects of membrane physiology that are discussed in this paper demonstrate the need for much more detailed knowledge of the 'target' before a rigorous interpretation of the effects of pressure can be achieved. From chemical kinetics it is known that high pressure lacks the unidirectional effect often found with temperature changes, and it is, therefore, not surprising to find an apparently haphazard mixture of effects on rates and equilibrium states in membrane-based reactions under pressure. The resolution of the critical, rate-determining step in a membrane process that is perturbed by high pressure is a worthwhile end in itself, and it has added interest when it helps us to understand the mechanisms by which anaesthetics and drugs interact with pressure. Comparable molecular information is also required to understand the adaptations to high pressure that have evolved in animals and bacteria in Nature.

It is a pleasure to acknowledge the collaboration with Dr K. T. Wann in a series of high pressure experiments. The work involved our students: M. L. J. Ashford, A. C. Hall, A. A. Harper, W. MacNaughtan and S. E. Wilcock; with support from the S.E.R.C., the M.O.D. and Aberdeen University Medical Endowments.

REFERENCES

- Akers, T. K. & MacCarter, D. K. 1973 Effects of high pressure on α - and β -adrenergic receptors of the rabbit duodenum. *Aerospace Med.* **44**, 60–62.
- Ashford, M. L. J., Macdonald, A. G. & Wann, K. T. 1982a The effects of hydrostatic pressure on the spontaneous release of transmitter at the frog neuromuscular junction. *J. Physiol., Lond.* **333**, 531–543.
- Ashford, M. L. J., Macdonald, A. G. & Wann, K. T. 1982b The effects of hydrostatic pressure and octanol on the conductance change produced by a quantum of acetylcholine at the frog neuromuscular junction. In *Proceedings of the Eighth Annual Congress of the European Undersea Biomedical Society*, pp. 184–204. Lübeck-Travemünde.
- Barkai, G., Goldman, B., Mashiach, S. & Shinitzky, M. 1983 The effect of physiologically relevant pressures on the phase transition of liposomes containing dipalmitoyl phosphatidylcholine. *J. Colloid. Interface Sci.* **94**, 343–347.
- Berger, L. R. 1974 Enzyme kinetics, microbial respiration, and active transport at increased hydrostatic pressure. *Rev. phys. Chem., Japan*, Special Issue, pp. 639–642.

- Blazyk, J. F., Melchior, D. L. & Steim, J. M. 1975 An automated differential scanning dilatometer. *Ann. Biochem.* **68**, 586–599.
- Boggs, J. M., Yoong, T. & Hsia, J. C. 1976 Site and mechanism of anesthetic action 1. Effect of anesthetics and pressure on fluidity of spin labelled lipid vesicles. *Molec. Pharmac.* **12**, 127–135.
- Bronfman, M. & Beaufay, H. 1973 Alteration of subcellular organelles induced by compression. *Fedn Eur. biochem. Soc. Lett.* **36**, 163–168.
- Brouha, A., Pequeux, A., Schoffeniels, E. & Disteché, A. 1970 The effect of high hydrostatic pressure on the permeability characteristics of the isolated frog skin. *Biochim. biophys. Acta* **219**, 455–462.
- Bryant, H. J. & Blenkinship, J. E. 1979 Action potentials in single axons: effects of hyperbaric air and hydrostatic pressure. *J. appl. Physiol. respir. envir. exercise Physiol.* **47**, 561–567.
- Campenot, R. B. 1979 The effects of high hydrostatic pressure on transmission at the crustacean neuromuscular junction. *Comp. Biochem. Physiol.* **52 B**, 133–140.
- Cattell, M. 1936 The physiological effects of pressure. *Biol. Rev.* **11**, 441–475.
- Ceuterick, F., Heremans, K., De Smedt, H., Nieuwenhuysen, P. & Clauwaert, J. 1979 Dynamic light scattering measurements in the phase transitions of phospholipid vesicles. *Chem. Phys. Lett.* **62**, 341–343.
- Ceuterick, F., Peeters, J., Heremans, K., De Smedt, W. & Olbrechts, H. 1978 Effect of high pressure, detergents and phospholipase on the break in the Arrhenius plot of *Azotobacter* nitrogenase. *Eur. J. Biochem.* **87**, 401–407.
- Champey, P., Büschlen, S. & Guillain, F. 1981 Pressure-induced inactivation of sarcoplasmic reticulum adenosine triphosphatase during high-speed centrifugation. *Biochemistry, N.Y.* **20**, 1520–1524.
- Chin, J. H., Trudell, J. R. & Cohen, E. N. 1976 The compression-ordering and solubility-disordering effects of high pressure gases on phospholipid bilayers. *Life Sci.* **18**, 489–498.
- Chong, P. L.-G. & Cossins, A. R. 1983 A differential polarised phase fluorometric study of the effects of high hydrostatic pressure upon the fluidity of cellular membranes. *Biochemistry, N.Y.* **22**, 409–415.
- Chong, P. L.-G., Shinitzky, M., Cossins, A. & Weber, C. 1981 Pressure effects on lipid bilayers and biological membranes. *Biophys. J.* **33**, 161 a.
- Clegg, R. M., Elson, E. L. & Maxfield, B. W. 1975 A new technique for optical observation of the kinetics of chemical reactions perturbed by small pressure changes. *Biopolymers* **14**, 883–887.
- Colton, A. & Colton, J. S. 1982a An electrophysiological analysis of oxygen and pressure on synaptic transmission. *Brain Res.* **251**, 221–227.
- Colton, A. & Colton, J. S. 1982b Depression of inhibitory synaptic transmission by oxygen and pressure. *Physiologist, Wash.* **25**, 222.
- Conti, F., Fioravanti, R., Segal, J. R. & Stühmer, W. 1982a Pressure dependence of the sodium current of squid giant axon. *J. membrane Biol.* **69**, 23–34.
- Conti, F., Fioravanti, R., Segal, J. R. & Stühmer, W. 1982b Pressure dependence of the potassium currents of squid giant axon. *J. membrane Biol.* **69**, 35–40.
- Cossins, A. R. & Macdonald, A. G. 1982 The fluidity of the bilayer in membranes from deep-sea fish. *J. Physiol., Lond.* **332**, 64–65.
- De Kruyff, B., Demel, R. A., Slotboom, A. J., Van Deenen, L. L. M. & Rosenthal, A. F. 1973 The effect of the polar headgroup on the lipid-cholesterol interaction: a monolayer and differential scanning calorimetry study. *Biochim. biophys. Acta* **307**, 1–19.
- De Kruyff, B., Demel, R. A. & Van Deenen, L. L. M. 1972 The effect of cholesterol and epicholesterol incorporation on the permeability and on the phase transition of intact *Acholeplasma laidlawii* cell membranes and derived liposomes. *Biochim. biophys. Acta* **255**, 331–347.
- De Smedt, H., Borghgraef, R., Ceuterick, F. & Heremans, K. 1979 Pressure effects on lipid-protein interactions in $(\text{Na}^+ + \text{K}^+)\text{-ATP-ase}$. *Biochim. biophys. Acta* **556**, 479–489.
- Doubt, T. J. & Hogan, P. M. 1979 Action potential correlates of pressure-induced changes in cardiac conduction. *J. appl. Physiol. respir. envir. exercise Physiol.* **47**, 1169–1175.
- Ebbecke, U. & Schaefer, H. 1935 Über den Einfluss hoher Drucke auf den Aktionsstrom von Muskeln und Nerven. *Pflügers Arch. ges. Physiol.* **236**, 679–692.
- Finch, E. D. & Kiesow, L. A. 1979 Pressure, anaesthetics and membrane structure: a spin-probe study. *Undersea biomed. Res.* **6**, 41–45.
- Flamm, M., Okubo, T., Turro, N. & Schachter, D. 1982 Pressure dependence of pyrene excimer fluorescence in human erythrocyte membranes. *Biochim. biophys. Acta* **687**, 101–104.
- Gaber, B. P. & Peticolas, W. L. 1977 On the quantitative interpretation of biomembrane structure by Raman spectroscopy. *Biochim. biophys. Acta* **465**, 260–274.
- Galla, H. J. & Trudell, J. R. 1980 Asymmetric antagonistic effects of an inhalational anesthetic and high pressure on the phase transition temperature of dipalmitoyl phosphatidic acid bilayers. *Biochim. biophys. Acta* **599**, 336–340.
- Galla, H. J. & Trudell, J. R. 1980b Pressure-induced changes in the molecular organisation of a lipid-peptide complex. *Biochim. biophys. Acta* **602**, 522–530.
- Gause, E. M., Mendez, V. M. & Rowlands, J. R. 1974 A spin label study of the effects of hydrostatic pressure and temperature on cellular lipids. *Spectrosc. Lett.* **7**, 477–490.
- Goldinger, J. M., Kang, B. S., Chou, Y. E., Paganelli, C. V. & Hong, S. K. 1980 Effect of hydrostatic pressure on ion transport and metabolism in human erythrocytes. *J. appl. Physiol. respir. envir. exercise Physiol.* **49**, 224–231.

- Gruenewald, B., Blume, A. & Watanabe, F. 1980 Kinetic investigations on the phase transition of phospholipid bilayers. *Biochim. biophys. Acta* **597**, 41–52.
- Hall, A. C., Ellory, J. C. & Klein, R. A. 1982 Pressure and temperature effects on human red cell cation transport. *J. membrane Biol.* **68**, 47–56.
- Hall, A. C. & Macdonald, A. G. 1980 Hydrostatic pressure alters the sodium content of erythrocytes. *J. Physiol., Lond.* **305**, 108P.
- Harper, A. A., Macdonald, A. G. & Wann, K. T. 1981 The action of high hydrostatic pressure on the membrane currents of *Helix* neurones. *J. Physiol., Lond.* **311**, 325–333.
- Harris, R. D., Jacobs, M., Long, M. M. & Urry, O. W. 1976 A high pressure sample cell for circular dichroism studies. *Ann. Biochem.* **73**, 363–368.
- Henderson, J. V. & Gilbert, D. L. 1975 Slowing of ionic currents in the voltage clamped squid axon by high pressure. *Nature, Lond.* **258**, 351–352.
- Henderson, J. V., Lowenhaupt, M. T. & Gilbert, D. C. 1977 Helium pressure alteration of function in squid giant synapse. *Undersea biomed. Res.* **4**, 19–26.
- Heremans, K. 1982 High pressure effects on proteins and other biomolecules. *Ann. Rev. Biophys. Bioengng* **11**, 1–21.
- Heremans, K. & Wuytack, F. 1980 Pressure effect on the Arrhenius discontinuity in Ca²⁺-ATP-ase from sarcoplasmic reticulum. *Fedn Eur. biochem. Soc. Lett.* **117**, 161–163.
- Jähnig, F. 1979 Structural order of lipids and proteins in membranes: Evaluation of fluorescence anisotropy data. *Proc. natn. Acad. Sci. U.S.A.* **76**, 6361–6365.
- Johnson, F. J., Eyring, H. & Stover, B. 1974 *The theory of rate processes in biology and medicine*. New York: Wiley.
- Johnson, M. L., Winter, T. C. & Biltonen, R. L. 1983 The measurement of the kinetics of lipid phase transitions: A volume-perturbation kinetic calorimeter. *Ann. Biochem.* **128**, 1–6.
- Johnson, S. M. & Miller, K. W. 1975 The effect of pressure and the volume of activation on the monovalent cation and glucose permeabilities of liposomes of varying composition. *Biochim. biophys. Acta* **375**, 286–291.
- Kamaya, H., Ueda, I., Moore, P. & Eyring, H. 1979 Antagonism between high pressure and anaesthetics in the thermal phase-transition of dipalmitoyl phosphatidylcholine bilayer. *Biochim. biophys. Acta* **550**, 131–137.
- Kendig, J. J. 1981 Currents in a voltage clamped vertebrate neuron at hyperbaric pressure. In *Underwater Physiology*, vol. 7 (ed. A. J. Bachrach & M. M. Matzen), pp. 363–370. Bethesda, U.S.A.: Undersea Biomedical Society.
- Kendig, J. J. & Cohen, E. N. 1976 Neuromuscular function at hyperbaric pressures: pressure–anaesthetic interactions. *Am. J. Physiol.* **230**, 1244–1249.
- Kendig, J. J., Schneider, T. M. & Cohen, E. N. 1978 Pressure, temperature and repetitive impulse generation in crustacean axons. *J. appl. Physiol. respir. envir. exercise Physiol.* **45**, 742–746.
- Kendig, J. J., Trudell, J. R. & Cohen, E. N. 1975 Effects of pressure and anaesthetics on conduction and synaptic transmission. *J. pharmac. exp. Ther.* **195**, 216–224.
- Kita, Y., Bennett, L. J. & Miller, K. W. 1981 The partial molar volumes of anaesthetics in lipid bilayers. *Biochim. biophys. Acta* **647**, 130–139.
- Kitching, J. A. 1957 Effects of high hydrostatic pressure on *Actinophrys sol* (Heliozoa). *J. expl Biol.* **34**, 511–517.
- Lee, A. G., Birdsall, N. J. M., Metcalfe, J. C., Toon, P. A. & Warren, G. B. 1975 Clusters in Lipid bilayers and the interpretation of thermal effects in biological membranes. *Biochemistry, N.Y.* **13**, 3699–3705.
- Lentz, B. R., Freire, E. & Biltonen, R. L. 1978 Fluorescence and calorimetric studies of the phase transitions in phosphatidylcholine multilayers: Kinetics of the pre-transition. *Biochemistry, N.Y.* **17**, 4475–4480.
- Little, H. J. 1982 Effects of ketamine and of high pressure on the responses of γ -aminobutyric acid of the rat superior cervical ganglion *in vitro*. *Br. J. Pharm. Chemother.* **77**, 209–216.
- Liu, N. & Kay, R. L. 1977 Redetermination of the pressure dependence of the lipid bilayer phase transition. *Biochemistry, N.Y.* **16**, 3484–3486.
- Macdonald, A. G. 1978 A dilatometric investigation of the effects of general anaesthetics, alcohols and hydrostatic pressure on the phase transition in smectic mesophases of dipalmitoyl phosphatidylcholine. *Biochim. biophys. Acta* **507**, 26–37.
- Macdonald, A. G. & Cossins, A. R. 1983 Effects of pressure and pentanol on the phase transition in the membrane of *Acholeplasma laidlawii* B. *Biochim. biophys. Acta* **730**, 239–244.
- MacNaughtan, W. & Macdonald, A. G. 1980 Effects of gaseous anaesthetics and inert gases on the phase transition in smectic mesophases of dipalmitoyl phosphatidylcholine. *Biochim. biophys. Acta* **597**, 193–198.
- MacNaughtan, W. & Macdonald, A. G. 1982 Effects of pressure and pressure antagonists on the growth and membrane-bound ATP-ase of *Acholeplasma laidlawii* B. *Comp. biochem. Physiol.* **72A**, 405–414.
- Mabrey, S. & Sturtevant, J. M. 1976 Investigation of phase transitions of lipids and lipid mixtures by high sensitivity differential scanning calorimetry. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3862–3866.
- Marsland, D. A. 1938 The effects of high hydrostatic pressure upon cell division in *Arbacia* eggs. *J. cell comp. Physiol.* **12**, 57–70.
- Marsland, D. A. 1970 In *High pressure effects on cellular processes* (ed. A. M. Zimmerman), pp. 259–312. New York & London: Academic Press.
- Melchior, D. L., Morowitz, H. J., Sturtevant, J. M. & Tsong, T. Y. 1970 Characterisation of the plasma membrane of *Mycoplasma laidlawii*. *Biochim. biophys. Acta* **219**, 114–122.

- Melchior, D. L., Scavitto, F. J., Walsh, M. T. & Steim, J. M. 1977 Thermal techniques in biomembrane and lipoprotein research. *Thermochimica Acta* **18**, 43–71.
- Mountcastle, D. B., Biltonen, R. L. & Halsey, M. J. 1978 Effect of anaesthetics and pressure on the thermotropic behaviour of multilamellar dipalmitoyl phosphatidylcholine liposomes. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4906–4910.
- Müller, K., Ludermann, H.-D. & Jaenicke, R. 1981 Reconstitution of lactic dehydrogenase from pig heart after reversible high pressure dissociation. *Biochemistry, N.Y.* **20**, 5411–5416.
- Nagle, J. F. 1973 Lipid bilayer phase transition: density measurements and theory. *Proc. natn. Acad. Sci. U.S.A.* **70**, 3443–3444.
- Nagle, J. F. & Wilkinson, D. A. 1978 Lecithin bilayers. Density measurements and molecular interactions. *Biophys. J.* **23**, 159–175.
- Ornhagen, H. C. L. & Hogan, P. M. 1977 Hydrostatic pressure and mammalian cardiac-pacemaker function. *Undersea biomed. Res.* **4**, 347–358.
- Otter, T. & Salmon, E. D. 1979 Hydrostatic pressure reversibly blocks membrane control of ciliary motility in *Paramecium*. *Science, N.Y.* **206**, 358–361.
- Paciorek, J., Morild, E. & Onarheim, J. 1982 Erythrocyte morphological changes in men living at pressure up to 500 m SW for up to 34 days. In *Proceedings of the Eighth Annual Congress of the European Undersea Biomedical Society*, pp. 249–280. W. Germany: Lübeck-Travemünde.
- Parmentier, J. L., Shrivastav, B. B. & Bennett, P. B. 1981 Hydrostatic pressure reduces synaptic efficiency by inhibiting transmitter release. *Undersea biomed. Res.* **8**, 175–183.
- Parmentier, J. L., Shrivastav, B. B., Bennett, P. B. & Wilson, K. M. 1979 Effect of interaction of volatile anaesthetics and high hydrostatic pressure on central neurons. *Undersea biomed. Res.* **6**, 75–91.
- Paul, K. L. & Morita, R. Y. 1971 Effects of hydrostatic pressure and temperature on the uptake and respiration of amino acids by a facultatively psychrophilic marine bacterium. *J. Bact.* **108**, 835–843.
- Pequeux, A. 1976 Polarisation variations induced by high hydrostatic pressures in the isolated frog skin as related to the effect on passive ionic permeability and active Na⁺ transport. *J. exp. Biol.* **64**, 587–602.
- Pequeux, A. & Giles, R. 1977 Effect of high hydrostatic pressures on the movements of Na⁺, K⁺ and Cl⁻ in isolated eel gills. *Experientia* **33**, 46–48.
- Pequeux, A., Giles, R., Pilwat, G. & Zimmerman, U. 1980 Pressure-induced variations of K⁺-permeability as related to a possible reversible electrical breakdown in human erythrocytes. *Experientia* **36**, 565–566.
- Phillips, M. C., Williams, R. M. & Chapman, D. 1969 On the nature of hydrocarbon chain motions in lipid liquid crystals. *Chem. Phys. Lipids* **3**, 304–364.
- Plachy, W. Z. 1976 Lipid phase equilibria at high pressure. *Biophys. J.* **16**, 138a.
- Regnard, P. 1887 Les phénomènes de la vie sous les hautes pressions – La contraction musculaire. *C. r. Séanc. soc. Biol.* **39**, 265–269.
- Regnard, P. 1891 *Recherches expérimentales sur les conditions de la vie dans les Eaux*, pp. 1–500. Paris: Masson.
- Reinhert, J. C. & Steim, J. M. 1970 Calorimetric detection of a membrane lipid phase transition in living cells. *Science, N.Y.* **168**, 1580–1582.
- Roer, R. D. & Shelton, M. G. 1982 Effects of hydrostatic pressure on Na transport in the freshwater crayfish, *Procambarus clarkii*. *Comp. biochem. Physiol.* **71 A**, 271–276.
- Sauter, J.-F. 1979 Electrophysiological activity of a mammalian sympathetic ganglion under hydrostatic and inert gas pressure. *Neuropharmacology* **18**, 77–81.
- Sauter, J.-F., Braswell, P., Wankowicz, P. & Miller, K. W. 1981 The effects of high pressures of inert gases on cholinergic receptor binding and function. In *Underwater Physiology*, vol. 7 (ed. A. J. Bachrach & M. M. Matzen), pp. 629–638. Bethesda, Md: Undersea Biomedical Society.
- Schlämm, N. A. & Daily, O. P. 1971 Uptake of B-galactosides by *Escherichia coli* at elevated pressure. *J. Bact.* **105**, 1202–1204.
- Segal, J. R. 1977 Pressure jump relaxation kinetics of frog skin open circuit voltage and short circuit current. *Biochim. biophys. Acta* **471**, 453–465.
- Shrivastav, B. B., Parmentier, J. L. & Bennett, P. B. 1981 A quantitative description of pressure induced alterations in ionic channels of the squid giant axon. In *Underwater Physiology*, vol. 7 (ed. A. J. Bachrach & M. M. Matzen), pp. 611–620. Bethesda, Md: Undersea Biomedical Society.
- Sleigh, M. A. & Macdonald, A. G. 1972 The effects of pressure on organisms. *Symp. Soc. exp. Biol.* vol. 26 (516 pages). Cambridge University Press.
- Spyropoulos, C. S. 1957 The effects of hydrostatic pressure upon the normal and narcotised nerve fibre. *J. gen. Physiol.* **40**, 849–857.
- Stamatoff, J., Guillon, D., Powers, L. & Cladis, P. 1978 X-ray diffraction measurements of dipalmitoyl phosphatidylcholine as a function of pressure. *Biochem. biophys. Res. Commun.* **85**, 724–728.
- Swezey, R. R. & Somero, G. N. 1982 Polymerization thermodynamics and structural stabilities of skeletal muscle actins from vertebrates adapted to different temperatures and hydrostatic pressures. *Biochemistry, Philad* **21**, 4496–4503.
- Taylor, R. F. & Robertson, R. F. 1980 Effects of normo- and hyperbaric pressure on an acetylcholine-binding proteoglycolipid from rat gastrocnemius tissue. *J. Neurochem.* **34**, 1166–1174.

- Trauble, H. 1971 The movement of molecules across lipid membranes: a molecular theory. *J. membrane Biol.* **4**, 193–208.
- Trauble, H. & Haynes, D. H. 1971 The volume change in lipid bilayer lamellae at the crystalline–liquid crystalline phase transition. *Chem. Phys. Lipids* **7**, 324–335.
- Trudell, J. R., Payan, D. G., Chin, J. H. & Cohen, E. N. 1974a The effect of pressure on the phase diagram of mixed dipalmitoyl-dimyristoylphosphatidylcholine bilayers. *Biochim. biophys. Acta* **373**, 141–144.
- Trudell, J. R., Payan, D. G., Chin, J. H. & Cohen, E. N. 1974b Pressure induced elevation of phase transition temperature in dipalmitoyl phosphatidylcholine bilayers. *Biochim. biophys. Acta* **373**, 436–443.
- Turro, N. J. & Okubo, T. 1981 Micellar microviscosity of ionic surfactants under high pressure. *J. Am. chem. Soc.* **103**, 7224–7228.
- Van Nice, P. S. & Galey, W. R. 1980 Actions of hyperbaric elemental gases on electrolyte transport in the human erythrocyte. *J. appl. Physiol. respir. Envir. exercise Physiol.* **49**, 204–210.
- Wann, K. T., Ashford, M. L. J. & Macdonald, A. G. 1980 Effect of alcohol anaesthetics and high hydrostatic pressure on spontaneous activity at the neuromuscular junction. In *Molecular mechanisms of anaesthesia. Progress in anaesthesiology*, vol. 2 (ed. B. R. Fink). New York: Raven Press.
- Wann, K. T., Harper, A. A., Wilcock, S. E. & Macdonald, A. G. 1977 Excitatory and inhibitory effects of pressure on neuronal membranes. *Med. subaqua. Hyperbare* **16**, 284–286.
- Wann, K. T. & Macdonald, A. G. 1980 The effects of pressure on excitable cells. *Comp. Biochem. Physiol.* **66A**, 1–12.
- Wann, K. T., Macdonald, A. G. & Harper, A. A. 1979 The effects of high hydrostatic pressure on the electrical characteristics of *Helix* neurons. *Comp. Biochem. Physiol.* **64 A**, 149–159.
- Wattiaux-De Coninck, S., Dubois, F., Mertens-Strijthagen, J., De Schrijver, C. & Wattiaux, R. 1980 Permeability of mitochondria to sucrose induced by hydrostatic pressure. *Biochim. biophys. Acta* **600**, 173–184.
- Wattiaux-De Coninck, S., Dubois, F. & Wattiaux, R. 1977 Lateral phase separation and structural integrity of the inner membrane of rat liver mitochondria. *Biochim. biophys. Acta* **471**, 421–435.
- Wilkinson, D. A. & Nagle, J. F. 1978 A differential dilatometer. *Ann. Biochem.* **84**, 263–271.
- Wong, P. T. T., Murphy, W. F. & Mantsch, H. H. 1982 Pressure effects on the Raman spectra of phospholipid membranes: pressure induced phase transitions and structural changes in 1,2-dimyristoyl 3-sn-phosphatidylcholine water dispersions. *J. chem. Phys.* **76**, 5230–5237.
- Woodhouse, B. J. 1976 Biological membranes at high pressure (ed. A. G. Macdonald & K. W. Miller). In *Biochemical and biophysical perspectives in marine biology*, vol. 3 (ed. D. C. Malim & J. R. Sargent) (447 pages). New York: Academic Press.
- Yager, P. & Peticolas, W. L. 1980 Statistical mechanical analysis of Raman spectroscopic order parameter changes in pressure-induced lipid bilayer phase transitions. *Biophys. J.* **31**, 359–370.
- Yager, P. & Peticolas, W. L. 1982 The kinetics of the main phase transition of aqueous dispersions of phospholipids induced by pressure jump and monitored by Raman spectroscopy. *Biochim. biophys. Acta* **688**, 775–785.